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January 21, 2003

Daniel E. Troy, Esq.
Chief Counsel
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

Re: Periostat® doxycycline hyclate 20 mg

Dear Mr. Troy:

During our recent discussion, you invited our client, CollaGenex Pharmaceuticals, Inc. ("CollaGenex" or the "Company"), which markets Periostat, to provide a written explanation of why Periostat is not subject to the Food and Drug Administration Modernization Act of 1997 ("FDAMA") antibiotic transition provisions and why it should receive both five year exclusivity and Hatch-Waxman patent protection under the Food, Drug, and Cosmetic Act ("FDCA" or "the statute"). As you requested, we are submitting CollaGenex's request in letter form rather than as a citizen petition and related petition for stay of action.

This letter makes four related requests. First, CollaGenex requests that the Food and Drug Administration ("FDA" or "the Agency") determine that Periostat does not contain an antibiotic drug as that term is defined in the FDCA, and that it is therefore not subject to the FDAMA antibiotic transition provisions. Second, CollaGenex requests that, as a consequence of the first finding, FDA find that, at approval, Periostat was eligible for five year exclusivity and that FDA recognize the remainder of that exclusivity. Third, CollaGenex requests, also as a consequence of the first finding, that FDA find that Hatch-Waxman patent protections apply to Periostat, with the result that CollaGenex, which has sued West-Ward Pharmaceuticals Corp. ("West-Ward") for patent infringement, was entitled to a thirty month stay in the consideration of West-Ward's abbreviated new drug application ("ANDA"). CollaGenex requests that FDA recognize the remainder of the stay.[1] Finally, CollaGenex requests that FDA not act on the pending West-Ward ANDA until FDA has resolved these issues in CollaGenex's favor or, if FDA finds against CollaGenex, until CollaGenex has been provided FDA's decision and has had at least ten business days to determine whether it will pursue alternative remedies.

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1. A finding that Hatch-Waxman patent protections apply would also result in disapproval of the West-Ward ANDA for failure to include appropriate patent certifications.

Factual Background

CollaGenex is a small pharmaceutical company that markets only one product, Periostat. Periostat (doxycycline hyclate 20 mg) is a prescription drug approved by FDA for use as an adjunct to scaling and root planing to promote attachment level gain and to reduce pocket depth in patients with adult periodontitis. To date, virtually all of the company's research and development budget has been devoted to research and development on the periodontitis indication and new indications for Periostat, which shows promise in a number of other areas. Thus, CollaGenex has made a profit during only one quarter – the most recent quarter - of its ten year existence.

The history of the current debate is as follows. CollaGenex acquired the rights to Periostat in 1992. At that time, Periostat was the subject of an Investigational New Drug Exemption ("IND") and no New Drug Application ("NDA") had been submitted. Building on the work of the previous IND holder, the Company spent ten years developing the product at a cost of over $20 million. On August 30, 1996, CollaGenex submitted an NDA for Periostat under section 505 of the FDCA ("section 505"), including listing information on CollaGenex's patents. The NDA was submitted under the number 20-642, which had been previously assigned by FDA when CollaGenex had submitted one section of the NDA in June 1996. Numbers in the 20-000 series are reserved for non-antibiotic drugs. On September 16, 1996, FDA staff called Christopher Powala at CollaGenex requesting that CollaGenex amend its submission to make it a submission under section 507 of the FDCA ("section 507"), which, at that time, governed the approval of antibiotic drugs. FDA also said that it intended to renumber the application to designate it NDA 50-744, an NDA number in the series reserved for antibiotic applications.

In 1996, antibiotics were not eligible for market exclusivity or for patent protection under the Hatch-Waxman provisions of the FDCA ("Hatch-Waxman"). FDA's request that Periostat be submitted to FDA as an antibiotic was, therefore, a matter of importance to CollaGenex. Mr. Powala responded to FDA's request by explaining to FDA staff that Periostat did not meet the definition of antibiotic in the statute, that it does not have antibiotic activity, that the company did not believe that it should be treated as an antibiotic, and that CollaGenex objected to amending its submission to make the product subject to section 507. FDA staff explained to Mr. Powala that, if the company failed to follow FDA's direction to amend its submission, the filing date of the NDA would be delayed while the issue was sorted out and that amending the Periostat application to submit under section 507 did not preclude CollaGenex from continuing to pursue the Company's objection to antibiotic status. Fearing an extensive delay that it could ill afford, CollaGenex agreed under protest to make the change requested by FDA and stated the Company's intention to continue to pursue the matter.

During the course of FDA's review of the NDA, CollaGenex continued to try to engage FDA in discussion of the antibiotic issue. Having been unable to locate anyone in the Center for Drug Evaluation and Research ("CDER") who would address the issue on the merits, on September 11, 1997, CollaGenex submitted a Request for Designation to the FDA

Ombudsman, which is attached as Exhibit A. Although CollaGenex tried to follow up, efforts to discuss the issue and/or to obtain a response were unsuccessful.

In November 1997, FDAMA abolished section 507 and made antibiotics subject to the section 505 drug approval process, thus providing antibiotics with eligibility for Hatch-Waxman market exclusivity and patent protections. At the same time, Congress concluded that antibiotic transition provisions were needed to ease the transition to section 505. In general, the transition provisions provide that products that contain an antibiotic drug that was the subject of an application under 507 are not eligible to receive market exclusivity or Hatch-Waxman patent protection.

By June 1998, CollaGenex had still received no determination from the Ombudsman. Knowing that FDA was close to approving Periostat, and at the suggestion of CDER's Ombudsman, CollaGenex renewed its request on July 8, 1998, this time with Dr. Murray Lumpkin of CDER. The request is attached as Exhibit B. In early September 1998, Dr. Lumpkin scheduled a telephone call to discuss the issues, in which he stated that FDA was of the opinion that Periostat was an antibiotic. On October 1, 1998, FDA approved Periostat under section 505. The approval letter, attached as Exhibit C, states that Periostat is subject to the FDAMA antibiotic transition provision.

FDA did not provide any explanation of its decision to apply the antibiotic transition provision. In fact, FDA has never provided written responses to any of CollaGenex's communications on this subject.

Having preserved its objection on the record, CollaGenex had no need to continue to contest FDA's treatment of Periostat as an antibiotic through litigation. At that time, and until very recently, CollaGenex believed that its strong patent position would protect it from generic competition for Periostat, and thought it unnecessary to waste its own, FDA's, and potentially the judiciary's resources to pursue an issue that was unlikely to make any difference as a practical matter.

Recently, however, CollaGenex learned that West-Ward had filed an ANDA for doxycycline hyclate 20 mg, listing Periostat as the reference drug. In correspondence with CollaGenex regarding CollaGenex's patent, West-Ward has stated that CollaGenex's patent is invalid or not infringed by the West-Ward product and that it intends vigorously to pursue a NDA approval. CollaGenex then instituted patent infringement litigation against West-Ward to protect the Company's patent rights. That litigation is pending.

If FDA had not applied the FDAMA antibiotic transition provision to Periostat and CollaGenex had received the market exclusivity and Hatch-Waxman patent protection to which it believes it is entitled, FDA would be precluded from approving the West-Ward ANDA for some time. Under current circumstances, however, FDA could and, in fact, must approve the West-Ward ANDA if the application meets the statutory criteria for approval. If FDA were to

approve the ANDA, West-Ward could market its product immediately, which would have a devastating impact on CollaGenex.

For these reasons, CollaGenex requested a meeting with you to renew its request that FDA determine that CollaGenex is not subject to the FDAMA antibiotic transition provisions. After discussion, you suggested that CollaGenex submit its request in a letter. In addition to explaining why CollaGenex believes that Periostat is not an antibiotic drug, the specific issues raised at the meeting are addressed.

Periostat Does Not Contain an Antibiotic Drug

The FDAMA antibiotic transition provision states that Hatch-Waxman exclusivity and patent protections will not apply to an NDA in which "the drug that is the subject of the application contains an antibiotic drug and the antibiotic drug was the subject of any application for marketing received by [FDA] under section 507 of [the FDCA] before the date of enactment of [FDAMA]."[2] Because Periostat does not contain an antibiotic drug, the transition provision does not apply.

Doxycycline Hyclate 20 mg Has No Antibiotic Effect

It is quite clear that 20 mg of doxycycline hyclate, the dosage of active ingredient in Periostat, does not function as an antibiotic.[3] There is no evidence whatsoever that it destroys or inhibits micro-organisms. FDA has acknowledged as much in the approved labeling for Periostat, which states that "[t]he dosage of doxycycline achieved with this product during administration is well below the concentration required to inhibit micro-organisms commonly associated with adult periodontitis" and "[t]his product should not be used for reducing the numbers of or eliminating those micro-organisms associated with periodontitis."[4]

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2. FDAMA, Pub. L. No. 105-115, § 125(d)(2), 111 Stat. 2295, 2327 (1997).

3. Periostat achieves its intended effects of promoting attachment level gain and reducing pocket depth in patients with adult periodontitis by inhibiting metalloproteinase (collagenase, gelatinase) enzymes that cause connective tissue breakdown.

4. See also, Dental Officer's Review of NDA 50-744, Clarence C. Gilkes, D.D.S. at 1 (Aug. 17, 1997) (Periostat "not antimicrobial at this dosage"); Review and Evaluation of Pharmacology and Toxicology Data (Jan. 4, 1997) (proposed dosage for Periostat is apparently below the threshold for antibiotic effects); Clinical Microbiology Review (May 15, 1997) (some topics routinely included in microbiology review for antibiotics considered unnecessary). Despite the apparent consensus that Periostat is not antimicrobial, FDA insisted on including in the labeling several precautions related to antimicrobial effects that CollaGenex understands are routinely included in labeling for the tetracycline class of products.

FDA's decision regarding the labeling was supported by a number of studies CollaGenex submitted during the NDA phase, all of which conclude that at 20 mg the quantity of doxycycline hyclate in Periostat is insufficient to exert an antimicrobial effect. The studies are summarized in Exhibit D. Three of those clinical studies also included an assessment of the development of resistance to low dose doxycycline, and, again, demonstrated that low dose doxycycline hyclate administration was not associated with the development of resistance, nor any cross-resistance with penicillin, ampicillin, cefoxitin, crythromycin, tetracycline, or metronidazole. The absence of any effect on the development of resistance is strong evidence that doxycycline hyclate 20 mg is not antimicrobial.[5]

Following the approval of Periostat an additional study was conducted specifically to answer the question of whether subantimicrobial levels of doxycycline would exert a detrimental effect on subgingival flora. The study concluded that no antimicrobial effect resulted during or following a treatment regime with 20 mg doxycycline bid.[6] Other studies similarly confirmed earlier research indicating that long term low dose doxycycline does not alter or contribute to alterations in the antibiotic susceptibility of subgingival microflora compared with placebo.[7] Thus, not only does the quantity of doxycycline in Periostat exert no antimicrobial effect, it does not contribute to changes in antibiotic susceptibility.

An additional study was designed specifically to determine whether doxycycline had an effect on intestinal or vaginal flora. The study analyzed stool specimens and vaginal swabs for total anaerobic counts, opportunistic pathogens, and doxycycline resistance from 70 subjects randomized to receive doxycycline or placebo and concluded there was no evidence that a nine month 20 mg regime of doxycycline exerted an effect on the composition or resistance level of either fecal or vaginal microflora.[8] The same conclusion resulted from a similar study

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5. When an antibiotic affects microorganisms, non-resistant organisms are inhibited, resistant organisms flourish, and the proportion of resistant organisms tends to increase. Thus the absence of an increase in resistant microorganisms demonstrates that an agent has no antimicrobial effect.

6. Walker C, Thomas J, Nangó S, et al. Long-Term Treatment with Subantimicrobial Dose Doxycycline Exerts No Antibacterial Effect on the Subgingival Microflora Associated with Adult Periodontitis, J Peridontol 2000;71:1465-1471. (Attached as Exhibit E)

7. Thomas J, Walker C, Bradshaw M. Long Term Use of Subantimicrobial Dose Doxycycline Does not Lead to Changes in Antimicrobial Susceptibility, J Periodontal 2000;71:1472-1483. (Attached as Exhibit F)

8. Walker C, Thomas J, Nangó S, Lennon J, et al. Effect of Sub-antimicrobial Dose Doxycycline (SDD) on Intestingal and Vaginal Flora, J Dent Res, IADR Abstracts 2000 (Attached as Exhibit G.)

analyzing the effects of doxycycline on skin flora. A six month regimen of 20 mg doxycycline b.i.d. exerted no detectable effect, either statistical or microbially, on the microflora of the skin relative to either baseline values or placebo values.[9]

At our recent meeting, one of the FDA participants suggested that Periostat might have antibiotic effect on micro-organisms not associated with periodontitis, referencing pharmacokinetic data showing steady state mean maximum doxycycline plasma levels of .79 micrograms/mL after Periostat administration, which he believed would be adequate to kill certain micro-organisms. He failed to note, however, distribution data, also referenced in the package insert, showing that doxycycline is greater than 90% bound to plasma proteins. Thus, only 10% is freely available; an effective level of .079 micrograms/mL in plasma, Periostat does not inhibit or destroy microorganisms even if they retain a profound susceptibility to doxycycline.[10]

The most profound evidence to establish that Periostat is not an antibiotic, however, are the human data themselves. CollaGenex has conducted a number of studies in more than 400 subjects to determine whether Periostat kills or inhibits micro-organisms. These were aimed at areas of the body known to have a high concentration of micro-organisms susceptible to doxycycline, including, the oral cavity, the skin, the gut and the genito-urinary tract. None of these studies shows an antibiotic effect, and they conclusively demonstrate that Periostat lacks antibiotic effect.[11]

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9. Clay Walker, Microbiology Report: Protocol # DERM-301, Efficacy of Dermostat (doxycycline hyclate) 20 mg tablets administrated twice daily for the treatment of acne (on file with author) (Attached as Exhibit H).

10. The lowest level of plasma concentration that results in antimicrobial levels in tissue is 1 microgram per milliliter. See McNamara TF, Golub LM, Ramamurthy N. Reduced Doxycycline Blood Levels in Humans Fail to Promote Resistant Organisms, presented at International Conference on Periodontal Disease: Pathogens & Host Immune Responses. Osaka, Japan, 1990.

11. It is notable that FDA's antibiotic regulations defined doxycycline hyclate as an antibiotic at 50, 100, or 300 milligrams of doxycycline, but did not state or even suggest that doxycycline hyclate 20 mg is an antibiotic. 21 C.F.R. § 446.120(a) (1995) ("Doxycycline hyclate capsules are composed of doxycycline hyclate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains doxycycline hyclate equivalent to either 50, 100, or 300 milligrams of doxycycline.") (regulation revoked September 24, 1998 as part of FDAMA implementation). Similarly, when FDA gave notice of doxycycline's uses as an antibiotic to treat anthrax, it specifically exempted doxycycline hyclate 20 mg. Prescription Drug Products: Doxycycline and Penicillin G Procaine Administration for Inhalational Anthrax (Post-Exposure), 66 Fed. Reg. 55679, 55680 (Nov. 2, 2001) (notice).

Periostat Does Not Fit The Statutory Definition of an Antibiotic Drug

At our meeting, FDA also suggested that, even if Periostat has no antibiotic effect, it is still an antibiotic, relying on an interpretation of the statutory definition of an antibiotic drug that would make non-antibiotic products into antibiotics as a matter of law.

It seems inconceivable that Congress intended its definition of antibiotic drug to capture products that do not kill microbes. Indeed, the very meaning of the word antibiotic – against life – suggests that an antibiotic is antimicrobial. Article after article in the scientific literature dating back to 1943 when the term antibiotic was first used, assumes, without discussion, that antibiotics have an injurious effect on the growth of microbes.[12] That is not surprising given the history of antibiotic development. The therapeutic application of antibiotic substances developed during the World War II era. Sir Alexander Fleming's accidental discovery of penicillin's lethal effect on microbes is well-known, and the "miracle" of antibiotics was in their ability to cure theretofore debilitating and lethal microbial infections.[13] Thus, the word "antibiotic" became synonomous with fighting infections.

FDA's own materials, as well as the scientific literature, reflect this understanding. In 1977, FDA's Advisory Review Panel on OTC Antimicrobial (II) Drug Products defined an antimicrobial, a category which includes antibiotics, as "an agent that kills or inhibits the growth and reproduction of micro-organisms."[14] When the monograph process begun by that

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12. See, e.g., Discussion between Dr. S.A. Waksman and Dr. J.E. Flynn on 19 January 1962, reproduced in J His Med, July 1973, at 285-6 ("let us make [antibiotic] into a noun which will include compounds that are produced by microbes which have an injurious effect upon the growth of other microbes"); Wesley W. Spink M.D., The Drama of Sulfanilamide, Penicillin and Other Antibiotics 1936-1972, Minnesota Medicine, June 1973, at 554-5 ("The discovery of penicillin and its successful application in the therapy of infections provided a stimulus to the search for similar antimicrobial agents . . ."); Selman A. Waksman, A Quarter-Century of the Antibiotic Era, Antimicrobial Agents and Chemotherapy, 1965, at 9 ("The results . . . have led to a better understanding of the production and utilization of certain metabolic products of microorganisms, known as antibiotics, for the treatment of infectious diseases.")

13. Spink, supra note 12, at 552-4.

14. Establishment of a Monograph for OTC Topical Antibiotic Products, 42 Fed. Reg. 17642, 17644 (April 1, 1977) (proposed rulemaking).

panel was completed with the adoption of a final rule on OTC antibiotics, all the permitted indications stated an antimicrobial effect.[15] As recently as 2000, in a preamble to a proposed rule, FDA equated the term "antibacterial drug products" with antibiotics.[16]

Instead of interpreting the statutory definition of an antibiotic to reflect this obvious meaning, however, FDA adopts a tortured interpretation that renders some of the words in the definition - "any quantity of" and "in dilute solution" – meaningless. The term "antibiotic drug" is defined in section 201(jj) of the FDCA and, in relevant part, reads as follows:

> The term "antibiotic drug" means any drug . . . intended for human use containing any quantity of any chemical substance which is produced by a micro-organism and which has the capacity to inhibit or destroy micro-organisms in dilute solution (including a chemically synthesized equivalent of any such substance) or any derivative thereof.[17]

One has only to read the definition to see that quantity matters – only a drug with any quantity of substance that will inhibit or destroy micro-organisms in dilute solution falls within its terms.[18] A drug that does not contain a dose of a substance that will inhibit or destroy micro-organisms in dilute solution cannot contain the requisite quantity.

Nevertheless, it appears that FDA reads the statute to mean that, if, at some dose, a substance will inhibit or destroy micro-organisms, then a product that contains that substance, no matter how small the dose, will be an antibiotic. That, however, is not a natural reading of the words. Had Congress intended to ignore the quantity of substance contained in the drug, it would doubtless have written a much simpler definition, deleting the words "any quantity of" altogether. FDA's interpretation renders the words "any quantity of" superfluous, which violates well established principles of statutory construction. The Supreme Court has made clear that a construction of a statute that renders a term "insignificant, if not wholly

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15. 21 C.F.R. § 333.150(b).

16. Labeling Requirements for Systemic Antibacterial Drug Products Intended for Human Use, 65 Fed. Reg. 56511 (Sept. 19, 2000) (proposed rulemaking) ("FDA is proposing to require that all systemic antibacterial drug products (i.e. antibiotics and their synthetic counterparts...")).

17. 21 U.S.C. § 321(jj).

18. The concept that dose matters is consistent with FDA's usual practice. New drugs are always evaluated at a particular dosage; NDAs are submitted for drugs at a particular strength; and FDA approves drugs at a particular dose. Given FDA's focus on dose, it is hard to understand why the Agency would choose to read this portion of the FDCA in a way that ignores dose and conflicts with standard agency practice.

superfluous" is impermissible, and it is "especially unwilling to do so when the term occupies so pivotal a place in the statutory scheme." Duncan v. Walker, 533 U.S. 167, 174 (2001). Further, construction of a statute that would result in Congress' inclusion of a word having "no operative effect on the scope of the provision" is not acceptable. Id. Yet this is exactly what FDA proposes to do.

It is particularly egregious to nullify not one but two statutory references to quantity in the antibiotic definition, which conditions antibiotic status not only on the "any quantity of" language but also on whether a substance will inhibit or destroy microorganisms "in dilute solution." Words in a statute must be interpreted with reference to the words that precede and follow them, and "the meaning of a word may be ascertained by reference to the meaning of words associated with it." Neal v. Clark, 95 U.S. 704, 708-9 (1877). See also Gregory v. Ashcroft, 501 U.S. 452, 465 (1991); Babbitt v. Sweet Home Chapter of Communities for a Great Oregon, 515 U.S. 687, 695 (1995). Here, the statutory reference to "dilute solution" reinforces the need to consider quantity. Even the most potent antimicrobial will not inhibit or destroy microorganisms if small enough quantities are used or if the solution in which it is placed is sufficiently dilute. Thus, whether or not a substance will inhibit or destroy microorganisms in dilute solution absolutely depends on the quantity of the substance that is placed in solution. By rendering the statutory reference to quantity meaningless, FDA also prevents the phrase "in dilute solution" from playing any meaningful role in the antibiotic definition.

In fact, FDA appears to ignore the words "in dilute solution" altogether. Certainly FDA has never explained publicly how it interprets the term, clarified how it determines an appropriate degree of dilution, or given any indication that it considers a substance's performance in dilute solution in defining antibiotic status. If CollaGenex's experience is representative, FDA instead goes out of its way to avoid having to explain itself. This is the third letter that CollaGenex has submitted to FDA on this subject, without having yet received a meaningful response. From an outsider's perspective, FDA appears to have employed an ad hoc approach to classifying drugs as antibiotics, presuming that it knows an antibiotic when it sees one. FDA has essentially interpreted the statutory definition of the term "antibiotic" in such a way that it makes no difference whether the two phrases "any quantity" and "in dilute solution" are present or absent. But this is not a permissible way to interpret a statute. FDA has a duty "to give effect, if possible to every clause and word of a statute," including, in this case, the references to "quantity" and "dilute solution." U.S. v. Menasche, 348 U.S. 528, 538-9 (1955), quoting Montclair v. Ramsdell, 107 U.S. 147, 152 (1883); Williams v. Taylor, 529 U.S. 362, 404 (2000) (describing this rule as a "cardinal principle of statutory construction").

FDA's interpretation might be more credible if FDA in the past had followed any logical pattern in applying it. But it has not. In one internal memo, Dr. Lumpkin, then Director of the Division of Anti-Infective Drug Products, stated that there is no "unambiguous Agency precedent on this matter" [i.e., distinguishing antibiotics from other drugs] and stated

that "[w]e all recognize the need for a consistent, defensible policy on this issue."[19] We have found no evidence, however, that the Agency heeded Dr. Lumpkin's call for a consistent defensible policy. Many biotechnology drugs are made by micro-organisms, and some may kill or inhibit micro-organisms at high enough concentrations. We have found no evidence, however, that FDA ever explored the issue with respect to biotechnology drugs made by micro-organisms or required the sponsors to determine whether these agents would kill micro-organisms. If FDA were actually applying its own definition, it would have required that every product produced by a micro-organism be tested to determine whether it will kill or inhibit micro-organisms.

Also, we are aware of several situations in which products should have, by FDA's interpretation, been treated as antibiotics but were not. For example, FDA apparently approved two NDAs for Lorabid (loracarbef) under section 505, even though the labeling states that the active chemical substance has antibiotic effect. The same appears to be true of Azactam (aztreonam), which was also submitted under section 505, but labeled as an antibiotic.

One need look no further than the Periostat labeling to demonstrate the illogic of FDA's interpretation. Having adopted a statutory construction that would make Periostat an antibiotic, it nevertheless felt compelled to point out in the approved labeling that the product should not be used to inhibit microorganisms. The inconsistency is too substantial to ignore.

In short, the only logical interpretation of the term antibiotic drug would preclude its application to Periostat. Moreover, in the past, FDA itself has failed to apply consistently the interpretation that it now advances. For these reasons, FDA cannot sustain its position that Periostat contains an antibiotic drug.

Patent Protection and Exclusivity Under Hatch-Waxman

Patent Protection: If FDA had not applied the FDAMA antibiotic transition provision to Periostat, a well-established statutory scheme would have applied. First, the patents that claim Periostat would have been listed in the Orange Book. West-Ward, in submitting its ANDA, would have been required to make one of four patent certifications. Given West-Ward's current statements that Periostat's patents are either invalid or not infringed by the West-Ward product, West-Ward presumably would have made a paragraph IV certification, which would have triggered a notice to CollaGenex of the ANDA submission. At that point, CollaGenex would have sued West-Ward (as it has now done), and a 30 month stay would have been in effect with respect to the West-Ward ANDA. CollaGenex is entitled to a 30 month stay.

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19. Memorandum from Murray M. Lumpkin, M.D., Director, Division of Anti-Infective Drug Products, to James Bilstad, M.D. and Bruce Burlington, M.D. (Nov. 26, 1990).

Exclusivity: As you know, newly approved drug products containing chemical entities not previously submitted under section 505(b) of the FDCA are entitled to five years of marketing exclusivity. To our knowledge, no previous doxycycline product has been submitted under section 505(b), and CollaGenex therefore was entitled to five years of exclusivity running from Periostat's approval date.

At our meeting, FDA counsel suggested that, as a result of the FDAMA antibiotic transition provision, all of the previously approved doxycycline products were submitted under section 505(b). By that logic, Periostat was not the first doxycycline product submitted under section 505(b) and is therefore ineligible for five year exclusivity.

The relevant transition provision states:

> An application that was approved . . . before [November 1997] for the marketing of an antibiotic drug under section 507 . . . shall . . . be considered to be an application that was submitted and filed under section 505(b) . . . and approved for safety and effectiveness under section 505(c) . . . except that if such application for marketing was in the form of an abbreviated application, the application shall be considered to have been filed and approved under section 505(j) . . .[20]

Because this provision plainly was intended to have only future effect, it does not convert old section 507 approvals into section 505(c) approvals. The purpose of the transition provision appears to have been to ensure the existence of a statutory framework for regulating previously-approved antibiotics after section 507 was abolished. Thus, the need was to cover these products in the future, not to address how these approvals were to be viewed in the past when section 507 existed and clearly applied. The drafter's intent to give the provision future rather than past effect is signaled in several ways in the statutory language. The provision states that old antibiotic applications "shall . . . be considered to be" 505(c) applications, which signals a present intention. Had the drafters intended the provision to have had past effect, they would have said that old antibiotic applications "shall . . . be considered to have been" 505(c) applications, which suggests applicability to the past. Also, the provision says that the old applications are to be "considered" 505(c) applications, not that they have become 505(c) applications. Had the drafters intended actually to convert these applications from the time they were issued, they would doubtless have said so.

In addition, reading the provision as FDA suggested at the meeting would create both redundancy and inconsistency with the portion of the "Exception" transition provision which immediately follows. That section exempts certain antibiotic drugs from Hatch-Waxman exclusivity provisions. If Congress had meant for all previous antibiotic drugs approvals to have become 505(c) approvals, it would not have needed a transition provision exempting antibiotic drugs containing a previously approved antibiotic from the five year exclusivity provisions, because they would already have been ineligible for five year exclusivity. In

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20. FDAMA, Pub. L. No. 105-115, § 125(d)(1), 111 Stats. 2295, 2326-27.

addition, reading the provision to have past effect would negate the portion of the "Exception" provision which refers to applications received under section 507. If all old applications are now considered submitted under 505, then they were certainly received under 505 as well. Thus, there would be no "application for marketing received by the Secretary of Health and Human Services under section 507 of such Act," which would negate the reference to old antibiotics in the "Exception" provision. If that were the correct reading, then all future antibiotics would be excluded from exclusivity and Hatch-Waxman patent protection, which is plainly not what the provision is intended to accomplish. For these reasons, we do not believe that the provision treating old 507 approvals as 505(c) approvals can be given retroactive effect.

Conclusion

Because Periostat is not an antibiotic, Hatch-Waxman exclusivity and patent protection should be provided to the extent that they would otherwise have existed, and we ask that FDA recognize and apply those provisions. We also ask that, pending FDA's consideration of this request, FDA not approve the West-Ward ANDA. CollaGenex has delayed filing a lawsuit in Federal Court solely to provide a period of time to resolve these issues without resort to litigation. It would be unjust for FDA to take advantage of CollaGenex's efforts to arrive at an amicable solution by approving the West-Ward ANDA while discussion is ongoing. We therefore request that, if FDA believes that it must approve the West-Ward ANDA imminently, it give CollaGenex at least ten business days notice so that CollaGenex will have the opportunity to initiate litigation on the issue before approval of the West-Ward ANDA.

Sincerely,

Nancy L Buc/Kate C Beardsley

Nancy L. Buc
Kate C. Beardsley

EXHIBIT A

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September 11, 1997

Ms. Amanda Bryce Norton
Chief Mediator and Ombudsman
Office of the Commissioner
Room 14-105, HF-7
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

BY HAND DELIVERY

Re: Periostat® NDA 50-774; Request for Designation

Dear Ms. Bryce Norton:

This request is submitted on behalf of our client, CollaGenex Pharmaceuticals, Inc. ("CollaGenex" or the "Company"). We hereby respectfully ask that the Food and Drug Administration ("FDA" or the "agency") designate the above referenced drug, which is the subject of a pending new drug application ("NDA"), as subject to the provisions of section 505(b) of the Federal Food, Drug, and Cosmetic Act ("FDC Act"), 21 U.S.C. § 355(b).

While we recognize this is not a typical designation request that is submitted under 21 C.F.R. Part 3, it nonetheless involves a significant product jurisdictional question appropriate for resolution by the Ombudsman's office. The precise issue addressed herein is whether Periostat® is properly subject to the antibiotic provisions of section 507 of the FDC Act, 21 U.S.C. § 357. In this regard, Periostat® does not meet the statutory definition of an "antibiotic drug." It is a synthetic drug that is neither intended for use as an antimicrobial drug product nor is it capable of inhibiting or destroying microorganisms at the dose levels that are utilized for periodontal disease. Therefore, Periostat® should not be subject to the antibiotic provisions of section 507 of the FDC Act.

Further in connection with this designation request, we respectfully request a waiver of 21 C.F.R. § 3.10, assuming the applicability of 21 C.F.R. Part 3 to this request. This provision provides that the application review clock is stayed during the pendancy of review by the product jurisdiction officer. Since this request does not pertain to which center(s) within FDA should have primary jurisdiction, but rather to which section of the FDC Act is pertinent to the approval of Periostat®, no reasons exist to stay the review of the pending NDA for Periostat® because of the submission of this designation request. Any decision in response to this petition will not affect jurisdiction of the Center for Drug Evaluation and Research ("CDER"), which is responsible for review of the NDA for Periostat®. We assume therefore that the waiver request has been granted upon the acceptance for filing of this designation request by FDA, unless we hear otherwise. Note that if this request is not granted upon acceptance of this petition for filing, then you should consider this submission withdrawn.

In accordance with 21 C.F.R. § 3.7, the following information is submitted:

IDENTITY OF SPONSOR

CollaGenex Pharmaceuticals, Inc.
301 S. State Street
Newton, PA 18940

Establishment Registration Number:	Not applicable.
Company Contact Person:	Mr. Christopher V. Powala Director, Drug Development & Regulatory Affairs
Telephone No.:	215-579-7388, extension 16
Facsimile No.:	215-579-8577

PRODUCT DESCRIPTION

Classification Name:

Not applicable.

Common, Generic, or Usual Name:

Doxycycline hyclate capsules USP (20 mg.)

Proprietary Name:

Periostat®.

Chemical, Physical, or Biological Composition:

Each Periostat® capsule is formulated to contain 20 mg of doxycycline hyclate USP as the only active ingredient.

Status and Brief Reports of Development Work:

With respect to the indicated use of doxycycline that is the subject of this request, in 1983, it was demonstrated that a semisynthetic tetracycline, minocycline, could inhibit collagen breakdown in the uncontrolled diabetic germ-free rat model of periodontal disease by a mechanism independent of its antimicrobial properties (Vol. 2.2, pp. 21-26). Further studies illustrated that this effect was achieved by blocking host-derived matrix metalloproteinases ("MMPs") (collagenase) and thus inhibiting bone and collagen loss. Animal studies have demonstrated that the tetracyclines, which have been chemically altered to render the molecule to be devoid of any anti-microbial activity, also

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Since it is impossible to include copies of all of the referenced information without exceeding the page limitations specified at 21 C.F.R. § 3.7(c), we are providing instead general citations to relevant volumes of the NDA 50-744 for Periostat®.

inhibit other matrix metalloproteinases, such as gelatinase and macrophage elastase, and thus can inhibit connective tissue destruction by a non-antimicrobial mechanism (Vol. 2.5, pp. 4-155). It also was found that doxycycline was the most potent inhibitor of MMPs of all the commercially available tetracyclines.

It has been shown in clinical studies that collagenase activity was reduced in gingival crevicular fluid as well as in adjacent gingival tissue after 14 days of 20 mg b.i.d. doxycycline hyclate administration (Vol. 2.109, pp. 1-8; 91-101). During a 12-week study evaluating the effects of doxycycline hyclate, 20 mg b.i.d. and placebo in patients with adult periodontitis, it was demonstrated that:

- No significant changes in gingival inflammation occurred, but there was a significant reduction of gingival crevicular fluid flow, an indication of MMP activity;
- Clinical parameters of tissue breakdown, *i.e.*, clinical attachment level and pocket depth, were significantly improved;
- Gingival crevicular fluid collagenase activity was statistically significantly reduced by 47.3 percent;

Description of Manufacturing Process:

CollaGenex relies on third-party contract manufacturers to produce doxycycline hyclate, the active ingredient in Periostat®, and to manufacture the finished dosage form (Vol. 1.1, CMC Section).

Proposed Use or Indications:

Periostat® is intended for use as a part of a professional oral health program to promote periodontal attachment gain and to reduce bone loss, pocket depth and bleeding on probing in patients with adult periodontal disease (Vol. 202, pp. 1-17).

Description of Modes of Action:

MMPs are an important family of zinc- and calcium-dependent endopeptidases secreted or released by a variety of host cells (*e.g.*, polymorphonucleocytes, macrophages, bone cells, and fibroblasts) that function at neutral pH and use the various constituents of the extracellular matrix as their substrates. These proteinases are involved in normal physiologic events such as bone remodeling and involution of the post-partum uterus. A variety of pathologic processes are characterized by elevated levels of MMPs, however, giving rise to increased connective tissue breakdown. These disease processes include rheumatoid and osteoarthritis, osteoporosis, and cancer metastasis. In particular, it has been shown that adult periodontitis is accompanied by increased levels of neutrophil collagenase in the gingival crevicular fluid.

Unlike existing treatments which focus on the bacterial infection associated with periodontitis, Periostat®, as a MMP inhibitor, disrupts the chronic progressive tissue degradation characteristic of the disease. As discussed in the Periostat® NDA (Vol. 2.2, pp. 21-26), the active ingredient in Periostat® (doxycycline hyclate) treats periodontitis by inhibiting matrix metalloproteinases (*i.e.*, leukocyte-type and fibroblast-type collagenase, gelatinase, and macrophage elastase) (Vol. 2.5, pp. 4-155). This mechanism of action is independent of the drug's antimicrobial properties at higher dosage levels (Vol. 2.18, pp. 1-50).

As also discussed in the Periostat® NDA, doses below 50 mg q.d. doxycycline hyclate are not effective in providing a measurable antibacterial effect (Vol. 2.18, pp. 1-50). The data and information submitted in support of the Periostat® NDA confirm that doxycycline hyclate at doses of 20 mg. q.d. or 20 mg b.i.d. provide a serum doxycycline concentration below the minimum 1.0 µg/mL doxycycline concentration (Vol. 2.2, p. 77). The results show that plasma concentrations were at a steady state by day 7 for the three treatment groups, with the mean pre-dose plasma doxycycline concentrations at steady state ranging from 0.13 to 0.14 µg/mL, 0.32 to 0.34 µg/mL, and 0.25 to 0.31 µg/mL following 20 mg q.d., 20 mg b.i.d., and 50 mg q.d. dosing, respectively. The mean steady state concentration and the mean steady state maximum concentration values following doxycycline hyclate treatments of 20 mg q.d. and

20 mg b.i.d. were all statistically significantly lower than 1.0µg/mL, the accepted threshold for antimicrobial activity.

Also, in terms of this request, nonclinical studies cited in the Periostat® NDA using culture plate analysis and speciation via DNA probe analysis showed no anti-bacterial effect of doxycycline hyclate 20 q.d. or 20 mg b.i.d. (Vol. 2.18, pp. 1-50 and Vol. 2.19, Report 5732.11F). No effects were observed on total anaerobic bacteria *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, or *Porphyromonas gingivalis*. *Fusobacteria*, or *Actinomyces* from the periodontium of patients with adult periodontitis.

Recent studies have shown that doxycycline and novel tetracycline analogs chemically modified to render them devoid of antimicrobial activity can inhibit connective tissue breakdown by a variety of direct and indirect mechanisms including (Vol. 2.5, p. 4; Vol. 2.2, pp. 21-26):

1. Direct, non-competitive inhibition of active collagenase, which appears to depend on the Ca++ and Zn++ binding properties of doxycycline;

2. Prevention of the conversion of pro-collagenase to collagenase, which appears to be independent of metal ion binding properties; and

3. Inhibition of the degradation of the serum protein, α_1-proteinase inhibitor.

Alpha$_1$-proteinase inhibitor is involved in the inhibition of other tissue destructive enzymes such as elastase which are not directly inhibited by doxycycline. Maintenance of high concentrations of α_1-proteinase inhibitor in tissue would protect elastase-susceptible connective tissue components such as elastic fibers, fibronectin, and proteoglycans, as well as maintaining high levels of the naturally occurring TIMPs (tissue inhibitors of metalloproteinases), which are also substrates for elastase.

Schedule and Duration of Use:

Periostat® is recommended for long-term daily use (up to one year) at dose level of 20 mg b.i.d.

Dose and Route of Administration:

Periostat® is intended solely for oral administration.

Description of Related Products and Regulatory Status:

Existing therapies and those treatments known by the Company to be under development for periodontitis are designed primarily to treat the bacterial infection associated with periodontitis on a short-term, periodic basis. These treatments include mechanical and surgical techniques, prophylactic approaches, such as mouthwashes, and locally delivered therapies.

We note that a variety of drugs indicated for antimicrobial use are sometimes regulated under section 507 of the FDC Act and sometimes not. These include metronidazole, which is subject to section 505. The precise basis for why some anti-infectives are classified as antibiotics and others are not is unclear. The agency appears to have been inconsistent in defining drugs that are subject to section 507.

Other Relevant Information:

By way of background, CollaGenex submitted to FDA the referenced pending NDA for Periostat® on August 30, 1996. The Periostat® NDA was accepted for filing on October 29, 1996. When CollaGenex originally submitted the application it was designated as NDA No. 20-642. On September 16, 1996, however, CDER's Division of Dermatologic and Dental Drug Products (the "Division") informed the Company that the NDA number had been changed to 50-744, a reflection of the fact that FDA assigns the 50,000-series numbers to full antibiotic applications. Nonetheless, the application is currently being reviewed by the Division of Dermatologic and Dental Drug Products, not the

Division of Anti-Infective Drug Products. Various FDA personnel have informed CollaGenex that its application is being handled and reviewed under section 507 of the FDC Act.

The Dental Drug Division advised CollaGenex when it filed the NDA that CollaGenex could request that the NDA be designated as a 505(b) application. The Company was also informed, however, that the submission of such a request at that time could significantly impede the agency's acceptance of the NDA for filing and substantive review. The Division also suggested that CollaGenex revise the applicable NDA cover letter and readdress the new drug/antibiotic designation issue once the NDA had been accepted for filing. Therefore, on September 17, 1996, CollaGenex submitted a revised cover letter and Form FDA 345h to reflect the new NDA number and to state that the NDA was submitted pursuant to section 507 of the FDC Act rather than section 505.[1] The Company is now addressing the antibiotic issue that is in dispute by the submission of this designation request. Although the agency component (CDER) is not in question, the product jurisdiction of Periostat® under section 507 is in dispute.

CollaGenex's Recommendation:

CollaGenex agrees that the agency component with primary jurisdiction for the review of the Periostat® NDA should be the Center for Drug Evaluation and Research, particularly the Division of Dermatologic and Dental Products, not the Division of Anti-Infective Drug Products. Given the mechanism of action of and the indicated use for the drug which is the subject of NDA 50-774, the Anti-Infective Division would not be the appropriate Division to review the subject NDA. CollaGenex also believes that the appropriate classification of its product is as a non-antibiotic drug subject to approval under section 505, not section 507, of the FDC Act, for the reasons discussed below.

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[1] Certain written correspondence that CollaGenex received from FDA regarding NDA 50-77 subsequent to that date states that the application was submitted pursuant to section 505(b) of the FDC Act. An action letter received on August 27, 1997, however, states that the NDA is not approvable under section 507 of the Act.

The relevant provisions pertaining to this recommendation are sections 201(g) and 507(a) of the FDC Act, 21 U.S.C. §§ 355(g) and 357(a). Section 201(g) is pertinent because although section 507(a) defines an antibiotic, it does so in the context of the use of the word "drug." Section 507 refers to "any drug . . . for use by man" that has certain characteristics further defined by section 507(a). Section 507 therefore cannot be read in isolation. It must be read in conjunction with section 201(g), which defines the term "drug" that is referenced in section 507.

In pertinent part, section 201(g) of the FDC Act defines the word "drug" to mean an article "intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease of man or other animals" (emphasis added). Therefore, whether a substance is a "drug" or "drug product" subject to section 507(a) depends on the product's intended use. FDA's regulations state that the words "intended use" or words of similar import refer to the objective intent of the manufacturer or other person legally responsible for the labeling of the product. 21 C.F.R. § 201.128 (1986). Objective intent can be shown by, among other things, labeling claims, advertising materials, or oral or written statements of such persons or their representatives. *Id.*

A product subcategory which meets the statutory definition of a "drug" in section 201(g) is an "antibiotic drug" if it also meets the requirements of section 507(a). Under the FDC Act all antibiotics described in section 507 are drugs if they meet the requirements of section 201(g), but not all drugs are antibiotics. The importance of this distinction traditionally is that antibiotics can be subject to certification and other requirements, whereas most other drugs are not. More relevant today is the consideration that although antibiotics are subject to abbreviated applications,[2] they are not subject to the exclusivity provisions of Title I of the Drug Price Competition and Patent Term Restoration Act of 1984 because they are not approved under section 505. See 57 Fed. Reg. 17950, 17951 (1992) and *Glaxo, Inc. v. Heckler*, 623 F. Supp. 69 (E.D.N.C. 1985).

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[2] See 21 C.F.R. § 314.92.

Section 507(a) of the FDC Act defines the term "antibiotic drug" to mean "any drug intended for use by man containing any quantity of any chemical substance which is produced by a microorganism and which has the capacity to inhibit or destroy microorganisms in dilute solution (including the chemically synthesized equivalent of any such substance)" (emphases added). It is unclear what the "intended for" language in section 507 adds, if anything, beyond that same language appearing in section 201(a) pertaining to the general definition of a drug. Thus, for a product to be categorized as an "antibiotic" drug, the rest of the language in section 507 states that two requirements must be met. The drug must both be produced by a microorganism (or be the synthetic equivalent thereof) and have the "capacity" to inhibit or destroy microorganisms "in dilute solution." In short, the definition is two-pronged, stating that status of a compound as an antibiotic is dependent both on its source or, in the case of a synthetic product, on its chemical structure, and its microbial activity in "dilute solution."

Periostat® does not meet the statutory "antibiotic drug" provisions of sections 201(a) and 507(a). It neither is intended for use as an antimicrobial agent nor does it actually have the capacity to inhibit or destroy microorganisms at the recommended dosage levels that are used to treat periodontitis. The clinical and nonclinical studies described in the "Mechanism of Action" section of the Periostat® NDA, which are reflective of objective intent, clearly demonstrate that the only active ingredient in the drug product, doxycycline hyclate, is for use in the treatment of periodontitis in a manner which is not dependent upon the inhibition or destruction of microorganisms.

In terms of the "source" aspect of the first prong of the antibiotic definition, doxycycline is synthetically produced and is not obtained from microbial sources. Periostat® does not contain any quantity of a drug derived from a microbe, particularly since microbes do not produce doxycycline. Further, doxycycline is not the "chemically synthesized equivalent" of oxytetracycline. Doxycycline is chemically different from oxytetracycline. Although doxycycline is derived from oxytetracycline, which is obtained from microorganisms, this fact should not trigger the source requirement of the definition. Section 507(a) does not state that any use of a microorganism to produce a drug renders the drug an antibiotic. For example, the use of a microorganism to produce an intermediate or a precursor of a drug, including active or inactive components, should not render the product an antibiotic. If it did, this interpretation

would ignore the actual language of the statute. Moreover, such an interpretation would require the agency to engage in a thorough investigation of the source of every component used in the manufacture of a drug, perhaps even for those that do not actually appear in the final drug product.

Undue emphasis on the "source" prong of the antibiotic definition can be problematic for other reasons. In this age of modern genetic techniques, microorganisms can produce a variety of substances such as hormones, insulin, and other drugs. Then, too, biological drugs that are regulated under section 351 of the Public Health Service Act, 42 U.S.C. § 262, could also be classified as antibiotics under this prong of the definition. *See* Intercenter Agreement Between the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research (CBER), at p. 5 (excepting products of cell culture from CBER regulation that are antibiotics). Further, although antibiotic regulation was established in 1945 when there was insufficient knowledge and control of fermentation processes and methods of analysis,[3] substantial advances in manufacturing and assay methods have occurred. The current lack of any certification requirements for antibiotics is testimony to these advancements. *See* 21 C.F.R. § 433.1 (1996). Indeed, the antibiotic provisions, as originally enacted, anticipated developments that would make antibiotic certification unnecessary. *See* Statement of Watson B. Miller, May 15, 1945, on H. Rept. No. 702, 79th Cong., 1st Sess., *reprinted in* Senate Reports, 79th Cong., 1st Sess., at p. 11. For this reason, provisions were enacted in 1945 and still are contained in the law today that allow for FDA to exempt antibiotic drugs from any of the requirements of section 507. *See* section 507(c), 21 U.S.C. § 357(c).

These and other considerations discussed below indicate that whatever relative importance the "source" prong of the antibiotic definition may once have had vis-à-vis the second prong of the definition, such importance seems to have waned considerably. The substantive and distinguishing aspect of the definition in section 507(a) therefore pertains to the second prong, the capacity of a drug to inhibit or destroy microorganisms "in dilute solution." Since this quoted language is not defined in the statute or in FDA's regulations, nor does there appear to be relevant legislative

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[3] *See, e.g.*, Senate Rep. No. 1744, Views of Senators E. McKinley Dirksen and Ramon L. Hruska, *reprinted in* 1962 U.S. Code Cong. & Adm. News 2884, 2926.

history on the topic, we can only presume what may have been intended. The language seems to refer to some inherent capacity of a chemical to exert an antimicrobial effect, even when "diluted." Many chemicals can have antimicrobial effects at "high" doses, whether derived from microorganisms or not. To repeat a trite, but relevant phrase, "The dose is the poison." In the present situation, we cannot help but feel therefore that this quoted language, coupled with the intended use language of section 201(a), is a reference to the dosage level at which drugs are administered. Indeed, even classical antibiotics, such as erythromycin or penicillin, will not inhibit or destroy microorganisms to any clinically significant degree if they are sufficiently diluted. Similarly, in the "dilute solution" of the recommended dosage levels of 20 mg b.i.d., Periostat® does not have the capacity to inhibit or destroy microorganisms.

Finally, we note also that the Clinton Administration and FDA in a report entitled "Reinventing the Regulation of Drugs and Medical Devices" (April, 1995) both are committed to repealing section 507. All antibiotics would formally be made subject to regulation under section 505. Indeed, the practical reality today is that antibiotics already are regulated like other drugs subject to section 505. We therefore wish to emphasize the significant competitive anomaly posed by section 507 status for Periostat®. Without Title I exclusivity, Periostat® will be subject to generic competition immediately upon publication of a relevant antibiotic monograph. CollaGenex has invested $14 million in the development of its drug for periodontal use. An adverse decision will enable competitors to copy Periostat® and will force CollaGenex to spend millions of dollars more in defending its patents covering Periostat®. It also will likely discourage further product innovation in the anti-infective area. The potential of these additional costs could prove devastating to CollaGenex as a small company.

In light of the foregoing facts and premises considered, Periostat® is not – and should not be treated as – an antibiotic drug within the meaning of sections 201(a) and 507(a) of the FDC Act. CollaGenex therefore respectfully requests that FDA designate the Periostat® NDA that has been accepted for filing by the Division of Dermatologic and Dental Drug Products as subject to the new drug provisions of section 505, not section 507, of the FDC Act.

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July 8, 1998

Confidential pursuant to 5 USC 552; 18 USC 1905; 21 USC 331 (j); 21 CFR 314.30 and 20.61.

Murray M. Lumpkin, M.D.
Deputy Center Director for Review Management
Center for Drug Evaluation & Research
1451 Rockville Pike, Rm. 6027 (HFD-001)
Rockville, MD 20852

Dear Dr. Lumpkin:

I am writing this letter on behalf of my client, CollaGenex Pharmaceuticals, Inc., to follow up on an earlier letter and my recent discussion with Mr. Morrison regarding the regulatory status of CollaGenex' product Periostat.® As you know, FDA has been reviewing CollaGenex' NDA for Periostat® as an antibiotic application under the now repealed section 507 of the Food, Drug, and Cosmetic Act ("FDCA"). CollaGenex believes that Periostat® should be approved under the new drug provisions in section 505 of the FDCA. The distinction is important because Periostat® will not be eligible for market exclusivity if it is approved as an antibiotic. Given the fact that Periostat® does not kill or inhibit microorganisms, it seems both counterintuitive and potentially confusing to treat it as an antibiotic. Further, there is no legal reason to do so; Periostat® does not fit the legal definition of an antibiotic because, among other reasons, it does not have the capacity to inhibit or destroy microorganisms. It seems particularly unnecessary to designate Periostat® as an antibiotic at a time when Congress has abolished the legal distinction between section 505 drugs and section 507 antibiotics. This letter explains why CollaGenex believes it is only appropriate to approve Periostat® under section 505.

Periostat® (doxycycline hyclate capsules, U.S.P., 20 mg.) is intended to be used as an adjunct to scaling and root planing to promote and maintain periodontal attachment level gain and to reduce pocket depth and bleeding on probing in patients with adult periodontal disease. It is recommended for long-term daily use (up to one year). Periostat® inhibits matrix metalloproteinases (collagenase, gelatinase, etc.), enzymes that cause connective tissue breakdown. Thus, it disrupts the chronic progressive tissue breakdown characteristic of periodontal disease.

Periostat® is not intended to nor does it destroy or inhibit microorganisms. To be sure, in dosages substantially higher than those in Periostat®, doxycycline has an antimicrobial effect, and doxycycline is approved for that use at dosages of 50 mg. twice daily and above. At the 20

mg. dosage in Periostat®, however, doxycycline does not destroy or inhibit microorganisms, providing a serum doxycycline concentration substantially below the minimum serum level of 1.0 microgram/ml needed for an antimicrobial effect. More information on studies of Periostat's® ability (actually, its lack thereof) to destroy or inhibit microorganisms has been provided previously in the Periostat® NDA and in the attached letter from Edward Korwek, submitted last September on CollaGenex' behalf. Also attached are abstracts of two forthcoming articles that provide additional information showing that Periostat® is not antimicrobial.

An NDA for Periostat® was submitted under section 505 in August 1996. The product was assigned for review to CDER's Division of Dermatologic and Dental Drug Products. Before filing the application, FDA requested that CollaGenex amend its cover letter to state that the application was being submitted under section 507. Although CollaGenex did not concur with FDA's determination that Periostat® is an antibiotic, the company submitted the revised cover letter, with the expressed intention of revisiting the designation issue at a later date. In September 1997, Mr. Korwek submitted the attached letter requesting that the Periostat® application be redesignated under section 505. During my recent conversation with Mr. Morrison, I agreed to renew in writing CollaGenex's previous request.

The FDCA defines an antibiotic as

> "any drug intended for use by man containing any quantity of a chemical substance which is produced by a microorganism and which has the capacity to inhibit or destroy microorganisms in dilute solution (including the chemically synthesized equivalent of any such substance)."[1]

The definition clearly contemplates that quantity matters. To be an antibiotic, a drug must contain a "quantity of a chemical substance ... which has the capacity to inhibit or destroy microorganisms in dilute solution." A quantity of drug that does not have the capacity to inhibit or destroy microorganisms would not fit the definition.[2] Thus, if Periostat® has the capacity to inhibit or destroy microorganisms in dilute solution, it is an antibiotic; otherwise, it is not. FDA has satisfied itself that doxycycline capsules containing 50, 100, or 300

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1. Former FDCA § 507(a); former 21 U.S.C. 357(a); now FDCA § 201(jj); 21 U.S.C. 321(jj).

2. An alternate reading, that the statute meant to encompass as an antibiotic a chemical substance if any quantity could destroy microorganisms, appears far less plausible. Had Congress meant that the law be interpreted this way, it could have eliminated the reference to quantity altogether so that the statute said that any drug containing a chemical substance produced by a microorganism and which has the capacity to inhibit microorganisms in dilute solution is an antibiotic. As a matter of statutory construction, the reference to quantity in the antibiotic definition has meaning only if it refers to the quantity in the drug at issue.

milligrams of doxycycline inhibit or destroy microorganisms in dilute solution; FDA's regulation establishing an antibiotic standard at these strengths makes that clear.[3] Periostat®, however, which contains doxycycline at a significantly lower strength, would not meet the test, in that at serum levels as administered according to Periostat's labeling, it will not kill or inhibit microorganisms even at full labeled strength, much less when diluted. Thus, even though doxycycline may be an antibiotic in some products, it is not an antibiotic in Periostat.®

Even if one were to conclude as a matter of law that Periostat® could fall within the definition of an antibiotic, FDA could, and in my view should, still decide to approve it under section 505. There are several precedents for doing so. One obvious example is preservatives. Although some products contain ingredients that would be antibiotics at a higher dosage level, when the same ingredient is used for preservative purposes, FDA does not treat the product as an antibiotic.[4] Similarly, both Lorabid® (loracarbef), approved in 1991, and Azactam (aztreonam), approved in 1986, which are the subject of antibiotic monographs, were approved under section 505.

Perhaps the best reason to treat Periostat® as a section 505 drug is common sense. Both medical professionals and consumers understand that antibiotics are products intended to destroy or inhibit microorganisms. Virtually every text we have identified proceeds on such assumptions. Stedman's medical dictionary, for example, defines antibiotic as "a soluble substance derived from a mold or bacterium that inhibits the growth of other microorganisms."[5] Similarly, Goodman and Gilman define antibiotic as a substance produced by various species of microorganisms that suppress the growth of other microorganisms and eventually may destroy them.[6] In the past, FDA has expressed the same view. One need look no further than the OTC rulemaking for Topical Antibiotic Products to see that this is the case. In its tentative final monograph, FDA interpreted the term antibiotic to refer to a product that has the capacity to inhibit or destroy microorganisms and concluded that "... it would be misleading to allow marketing of an antibiotic containing drug product without labeling that

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3. 21 CFR 446.120a. ("Doxycycline hyclate capsules are composed of doxycycline hyclate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsules. Each capsules contains doxycycline hyclate equivalent to either 50, 100, or 300 milligrams of doxycycline.") (regulation to be revoked September 24, 1998 as part of implementation of the FDA Modernization Act of 1997).

4. See, e.g., 21 CFR 433.22, Biologic drugs that contain antibiotics as preservatives (regulation to be revoked September 24, 1998 as part of implementation of the FDA Modernization Act of 1997).

5. Stedman's Medical Dictionary, 25th Edition (1990).

6. Goodman and Gilman, The Pharmacological Basis of Therapeutics, ninth edition, p. 1029.

indicates the product has microbial activity."[7] Treating Periostat® as an antibiotic when it has no antimicrobial effect would likewise be misleading.

The FDA Modernization Act of 1997 makes the common sense approach even stronger. Because the distinction between antibiotics and drugs has been eliminated, FDA need not be concerned about the precedential effect of its decision on this product or about whether it is effectuating the intent of the Congress. Both Congressional intent and the future treatment of antibiotic products is clear.

CollaGenex appreciates your willingness to look at this issue. I will call you shortly to follow up.

Sincerely,

Nancy L. Buc

cc: NDA 50-744

7. FDA. Topical Antimicrobial Drug Products for Over-the-Counter Human Use: Tentative Final Monograph. 47 Fed. Reg. 29986. 29988. 29991 (July 9. 1982).

EXHIBIT C

NDA 50-744

CollaGenex Pharmaceuticals, Inc.
Attention: Christopher Powala
Director, Drug Development and Regulatory Affairs
301 South State Street
Newtown, PA 18940

Dear Mr. Powala:

Please refer to your new drug application (NDA) dated August 30, 1996, received August 30, 1996, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Periostat™ (doxycycline hyclate USP) Capsules, 20 mg. We note that this application is subject to the exemption provisions contained in section 125(d)(2) of Title I of the FDA Modernization Act of 1997.

We acknowledge receipt of your submissions dated August 28, October 1, November 13, December 8, 1997; January 6, 14, and 19, February 10, March 2, 18, and 31, April 23 and 28, July 9 and 29, and September 3, 14, 16, 22, 24 (2), and 25, 1998. Your submission of March 31, 1998 constituted a full response to our August 27, 1997, action letter. The user fee goal date for this application is October 1, 1998.

This new drug application provides for the use of Periostat™ (doxycycline hyclate USP) Capsules, 20 mg as an adjunct to subgingival scaling and root planing to promote attachment level gain and to reduce pocket depth in patients with adult periodontitis.

We have completed the review of this application, as amended, and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed labeling text. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed labeling (text for the package insert, immediate container and carton labels). Marketing the product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug. We acknowledge your commitment made in the teleconference with this Division on September 16, 1998, to revise the carton and container labeling so that the prominence of the established name and tradename is commensurate and in accordance with 21 CFR 201.10(g)(2).

Please submit 20 copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy-weight paper or similar material. For administrative purposes, this submission should be designated "FPL for approved NDA 50-744". Approval of this submission by FDA is not required before the labeling is used.

We remind you of your Phase 4 commitments agreed to in your submissions dated August 3, 1998, and September 14, 1998. These commitments, respectively, are listed below:

Protocols, data, and final reports should be submitted to your IND for this product and a copy of the cover letter sent to this NDA. If an IND is not required to meet your Phase 4 commitments, please submit protocols, data and final reports to this NDA as correspondence. In addition, under 21 CFR 314.82(b)(2)(vii), we request that you include a status summary of each commitment in your annual report to this NDA. The status summary should include the number of patients entered in each clinical study, expected completion and submission dates, and any changes in plans since the last annual report. For administrative purposes, all submissions, including labeling supplements, relating to these Phase 4 commitments must be clearly designated "Phase 4 Commitments".

In addition, please submit three copies of the introductory promotional materials that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please submit one copy to this Division and two copies of both the promotional materials and the package insert directly to:

Division of Drug Marketing, Advertising, and Communications, HFD-40
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

Please submit one market package of the drug product when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, contact Roy Blay, Ph.D., Project Manager, at (301) 827-2020.

Sincerely,

Jonathan K. Wilkin, M.D.
Director
Division of Dermatologic and Dental Drug Products
Office of Drug Evaluation V
Center for Drug Evaluation and Research

Enclosure

EXHIBIT D

SUMMARY OF NDA STUDIES

Study No.	Method	Conclusion/Result	Pages *
C1-95-102	Open-label, repeated dose, randomized, three period cross-over study (20mg. q.d. /20mg. b.i.d./ 50mg. q.d.) of 30 subjects.	Plasma values of doxycycline were below threshold of antimicrobial activity at 20mg.	18-0008 to 18-0009
92-034	Randomized, multiple dose, three period, cross-over study (20mg. b.i.d./40mg. q.d./50mg b.i.d.) of 15 subjects.	The plasma values for 20mg bid and 40mg q.d. were statistically significantly below the threshold for antimicrobial activity.	18-0010 to 18-0012
5732.11A	Double-blind, placebo-controlled, randomized study of 66 subjects with adult periodontitis (20mg. doxycycline hyclate b.i.d/20 mg. q.d.). DNA Probe analysis.	No shift in levels of P. intermedia or P. gingivalis, (data insufficient data to support statistical analysis).	18-0014 to 18-0018; 19-0001 to 19-0008
5732.11E	Double-blind, placebo controlled parallel study (10mg. q.d./ 20mg b.i.d./ placebo bid) of 40 subjects.	Doxycycline hyclate doses of up to 20 mg. b.i.d. does not have an antibacterial effect on total anaerobic, fuso - bacterium or Actinomyces counts.	18-0018 to 18-0024; 19-0009 to 19-0035
5732.11F	40 subject, 12 month, double-blind, placebo-controlled, trial to evaluate the effects of low dose doxycycline on attachment levels (10mg. q.g/ 20mg. q.d., 20 mg. b.i.d.)	Doxycycline hyclate did not alter the population dynamics of bacterial species through antimicrobial action.	18-0024 to 18-0045; 19-0036 to 19-00193

* All page citations are to volumes 2-18 and 2-19 of CollaGenex Pharmacueticals, Inc., NDA 20-642 Submission, Section 7 – Microbiology (dated August 30, 1996).

Long-Term Treatment With Subantimicrobial Dose Doxycycline Exerts No Antibacterial Effect on the Subgingival Microflora Associated With Adult Periodontitis

Clay Walker,* John Thomas,† Sonia Nangó,* Jennifer Lennon,* Jeanne Wetzel,† and Christopher Powala‡

Background: The purpose of this study was to determine whether treatment with subantimicrobial dose doxycycline (SDD), 20 mg bid, exerted an antimicrobial effect on the microflora associated with adult periodontitis.

Methods: Following the approval of the protocol and informed consent forms by the respective IRBs at the University of Florida and West Virginia University, 76 subjects with adult periodontitis were entered and randomly assigned to receive SDD or placebo. A split-mouth design was utilized, with each subject receiving subgingival scaling and root planing (SRP) in two quadrants immediately following baseline data collection, while the remaining two quadrants were left unscaled (non-SRP). Microbial samples were collected prior to treatment, after 3, 6, and 9 months of treatment, and after 3 months of no treatment. The samples were examined by microscopy and by enumeration on selective and non-selective media.

Results: All treatments resulted in statistically significant decreases in the proportions of spirochetes and motile rods ($P < 0.05$) and in an increase in the proportion of coccoid forms ($P < 0.0001$) relative to baseline. No between-treatment differences were detected between the SDD and placebo treatments in either the SRP or non-SRP design, with the exception of the small and large spirochetal groups. The spirochetal proportions present in the SDD group were significantly lower ($P < 0.05$) than the paired placebo group during the 9-month treatment and was preceded by a significant decrease ($P < 0.01$) in the proportion of microbiologic sample sites that bled on probing. No between-treatment differences were detected in any of the other microbial parameters.

Conclusion: The microbial differences observed were attributed to the anticollagenase and anti-inflammatory properties of SDD and not to an antimicrobial effect. *J Periodontol 2000;71: 1465-1471.*

KEY WORDS

Periodontitis/microbiology; doxycycline/therapeutic use; clinical trials, controlled.

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Subantimicrobial dose doxycycline (SDD) consisting of 20 mg doxycycline hyclate§ bid has been approved as an adjunct to periodontal scaling and root planing (SRP) for the treatment of adult periodontitis. Doxycycline, like tetracycline and minocycline, in addition to being a broad-spectrum antimicrobial agent, also has inhibitory activity on host-derived collagenases and other matrix metalloproteinases by mechanisms independent of its antimicrobial properties. Specifically, tetracyclines inhibit the activity of mammalian neutrophil and osteoblast collagenases that appear crucial in the destruction of Type I and II collagen found in the periodontal ligament.[1,2] Apart from their anticollagenase activity, tetracyclines are also reported to have anti-inflammatory properties and to be potent inhibitors of osteoclast function.[3] Doxycycline is the most potent anticollagenase inhibitor of the commercially available tetracyclines with IC50 values of 16 to 20 μM for collagenases from PMNs, dental plaque, and gingival tissue.[4,5] Several short-term clinical studies have reported that SDD resulted in a decrease in collagenase activity which was accompanied by a beneficial and significant improvement in attachment

§ Periostat, CollaGenex Pharmaceuticals, Inc., Newtown, PA.

levels and probing depths.[6,7] More recently, a long-term, multi-centered clinical study compared the efficacy of a 9-month regimen of SDD following SRP to a placebo control and found that the use of SDD/SRP showed statistically significant improvements in attachment level and probing depth relative to SRP with a placebo.[8]

Substantial evidence indicates that the adjunctive use of SDD provides a significant benefit to SRP due to its anticollagenase and anti-inflammatory activities rather than to its antimicrobial activity. However, serious concern has been expressed that even subantimicrobial levels of doxycycline may exert a detrimental effect on the subgingival flora. Such an effect could result in the disruption or suppression of the normal flora and lead to its colonization or overgrowth by periodontal or opportunistic pathogens. The purpose of this study was to stringently evaluate the effects of a 9-month regimen of 20 mg doxycycline bid relative to a placebo control on the subgingival flora.

MATERIALS AND METHODS

Study Design

Clinical and microbial data were collected at the University of Florida and West Virginia University from subjects with adult periodontitis during a 9-month treatment period followed by a 3-month no-treatment period. Microbiological samples of subgingival plaque were collected prior to the initiation of treatment (baseline), after 3, 6, and 9 months of treatment, and at 3 months post-treatment. A total of 76 subjects (38 at each study site) with adult periodontitis who met the inclusion and exclusion criteria set forth in the experimental protocol were entered into the placebo-controlled, double-blind treatment phase.

The design of the study was as follows: A split-mouth design was used where two quadrants in each subject received scaling and root planing (SRP) while the opposite two quadrants did not (non-SRP). The quadrants selected to receive SRP were required to have a minimum of two sites with probing depth (PD) and loss of attachment level (AL) of ≥5 but ≤9 mm and that bled on probing. The non-SRP quadrants may or may not have met this criteria. Each subject was then randomly assigned to receive either SDD or placebo treatment. Thus, in effect, there were four treatment groups: SRP-SDD, non-SRP-SDD, SRP-placebo, and non-SRP-placebo. SRP-placebo was considered as a positive control, while non-SRP placebo was a true negative control. Thus, the study was considered to consist of two parallel experiments. SRP-SDD and non-SRP-SDD were paired as were non-SRP-SDD and non-SRP-placebo so that the SDD was the variable tested.

All subjects who completed the 9-month treatment phase were invited to continue in a 3-month no-treatment phase. Of the 67 subjects who completed the 9-month treatment phase, 27 of 36 and 26 of 29 subjects at the University of Florida and West Virginia University, respectively, returned for sampling at the end of the 3-month no-treatment period.

A total of 4 sites, distributed in a minimum of 3 quadrants (4 quadrants were selected where possible), with PD ≥5 mm but ≤8 mm were selected in each subject for microbial sampling; two sites were from the SRP quadrants and two from the non-SRP quadrants. These sample sites were retained throughout the study. Plaque samples were collected using sterile endodontic paper points as previously described.[9] The two microbial samples collected from the SRP sites were pooled by subject and then processed, as were the two samples from the non-SRP sites.

Microbial Enumeration

Immediately following collection, samples were transported to the microbiology laboratories. The samples were gently sonicated to dispense adherent plaque and then processed. Each sample was examined by direct microscopy and by culture on selective and non-selective media.

Microscopy. A 10 µl aliquot of the sample was removed under anaerobic conditions and placed on a clean slide for examination at 1,000× by dark-field microscopy. Eight distinct cellular morphotypes were distinguished and enumerated as previously described.[10]

Selective and non-selective media. Following a series of 10-fold dilutions in pre-reduced, anaerobic-sterilized Ringers solution, performed under strict anaerobic conditions, 0.1 ml aliquots were dispensed onto agar plates and spread with sterile glass rods. The following taxa were enumerated on selective and non-selective media: total anaerobic counts, total facultative counts, total *Streptococcus*, total *Actinomyces*, *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, enteric bacteria, *Staphylococcus aureus*, and *Candida*. Estimates of obligate anaerobic bacteria were determined by subtracting the total facultative count from the total anaerobic count. If the facultative count was greater than the anaerobic count, a zero value was entered for the obligate anaerobes. Bacteria tentatively identified as *P. intermedia* are, in reality, *P. intermedia sensu lato* since *P. intermedia* was not differentiated from *P. nigrescens*.

Statistical Analyses

The study was considered to consist of two parallel experiments, each of which was designed to test for differences between doxycycline treatment and a placebo control. One design sought for differences following conventional periodontal treatment consisting of mechanical scaling and root planing (SRP), and the second sought for differences without the initial peri-

odontal therapy of scaling and root planing (non-SRP). With this in mind, the resulting data sets were analyzed with the subject as the statistical unit to detect if differences existed at any sample period between doxycycline-treated and placebo-treated subjects.

The factoral ANOVA and Fisher's PLSD test were utilized to determine if statistically significant differences were present between the paired treatment groups at each sample period. The repeated measures ANOVA was used for longitudinal analyses to test for differences within a treatment. If differences were detected longitudinally, the paired *t* test was used to detect the location of the differences. In cases where outliers were suspected, e.g., microbial culture counts that could influence parametric analyses, the Wilcoxon signed rank, a non-parametric version of the paired *t* test, was used to verify statistical significance. Since the paired *t* test and Wilcoxon signed rank require matched samples from the same subject and the 3-month post-treatment data were derived from fewer subjects than the 9-month data set, it was necessary to construct a new data set limited to those subjects who consented to participate in the 3-month no-treatment phase for analyses seeking differences in the latter.

A total of 78 subjects were entered at the two study sites with the expectation that a minimum of 65 subjects would complete the 9-month treatment phase of the study. This sample size, if equally split, had a 90% power of detecting a difference of 1 $\log_{10}$ in microbial counts between SDD and the paired treatment. All statistical comparisons were based on $P \leq 0.05$.

RESULTS

Microscopic Enumeration

Differences between and within treatment groups were analyzed for each of the following morphological groups: small, intermediate, and large spirochetes; motile rods; coccoid forms; non-motile rods; fusiforms; and filamentous rods.

Between-treatment differences. No between-treatment differences were detected for any morphological group other than the spirochetes. In the SRP design, the proportion of small spirochetes (Table 1) present at the 3- and 6-month sample periods and the proportion of large spirochetes (Table 2) present at the 6-month sample were significantly lower in the SDD group than in the placebo group ($P<0.05$). In the non-SRP design, the proportions of both the small and large spirochetal groups present at the 9-month sample were significantly lower in the SDD group than in the placebo group ($P<0.05$).

Within-treatment differences. Differences within a treatment were analyzed using the paired *t* test to detect if the treatment had any significant effect on a particular morphologic group. Both the SDD and placebo treatments, regardless of SRP or non-SRP design, produced statistically significant reductions in both the intermediate and large spirochetal groups (Tables 2 and 3). In the SRP design, the SDD treatment yielded significant reductions in small spirochetes, relative to baseline, for all 9 months of treatment, while the placebo treatment demonstrated only significant reductions at the 9-month sample period (Table 1). Significant reductions in the proportion of motile rods were detected for all treatments at all sample periods relative to baseline (Table 4). Significant increases ($P<0.0001$) were found in the proportion of coccoid forms, relative to baseline, for all sample periods (Table 5). No significant changes were noted during any treat-

Table 1.

Mean Percentage of Small Spirochetes Relative to Total Microscopic Flora for SDD and Placebo Treatment Groups in SRP and Non-SRP Design

Design	Treatment Group	Baseline	3 Months	6 Months	9 Months Post
SRP	SDD	10.35	[illegible]	[illegible]	4.95†
SRP	Placebo	10.36	[illegible]	10.40*	5.89†
Non-SRP	SDD	9.98	[illegible]	[illegible]	6.58*
Non-SRP	Placebo	11.42	[illegible]	[illegible]	9.79*

* Statistically significant differences ($P \leq 0.05$) between SDD and placebo treatment groups.
† Statistically significant within-treatment differences ($P \leq 0.05$) relative to baseline.

Table 2.

Mean Percentage of Large Spirochetes Relative to Total Microscopic Flora for SDD and Placebo Treatment Groups in SRP and Non-SRP Design

Design	Treatment Group	Baseline	3 Months	6 Months	9 Months Post
SRP	SDD	3.34	[illegible]	[illegible]	0.58†
SRP	Placebo	4.29	[illegible]	[illegible]	1.74†
Non-SRP	SDD	3.22	[illegible]	[illegible]	0.56*†
Non-SRP	Placebo	3.13	[illegible]	[illegible]	1.79*†

* Statistically significant differences ($P \leq 0.05$) between SDD and placebo treatment groups.
† Statistically significant within-treatment differences ($P \leq 0.05$) relative to baseline.

ble 3.

Mean Percentage of Intermediate Spirochetes Relative to Total Microscopic Flora for SDD and Placebo Treatment Groups in SRP and Non-SRP Design

Design	Treatment Group	Baseline	Treatment Phase		
			3 Months	6 Months	9 Months Post
SRP	SDD	15.57	1.62*	1.88*	
	Placebo	13.43	3.77*	5.63*	
Non-SRP	SDD	13.56	2.85*	5.86*	
	Placebo	13.94	4.00*	6.33*	

* Statistically significant within-treatment differences ($P \leq 0.001$) relative to baseline.

Table 4.

Mean Percentage of Motile Rods Relative to Total Microscopic Flora for SDD and Placebo Treatment Groups in SRP and Non-SRP Design

Design	Treatment Group	Baseline	Treatment Phase		
			3 Months	6 Months	9 Months Post
SRP	SDD	6.93	2.79*	1.55*	
	Placebo	6.63	1.23*	1.93*	
Non-SRP	SDD	8.64	1.79*	2.61*	
	Placebo	6.53	3.66*	3.46*	

* Statistically significant within-treatment differences ($P \leq 0.05$) relative to baseline.

ment in the proportion of non-motile rods, fusiforms, or filamentous rods present at any sample period.

Culture Enumeration

As with the microscopic parameters, data analyses were conducted to detect statistically significant differences both between and within the treatment groups.

Between-treatment differences. With one single exception, no statistically significant differences ($P > 0.300$) were detected between SDD and placebo treatments in either the SRP or non-SRP design at any sample period for the total cultivable bacterial mass (total anaerobic counts, total facultative counts, or obligate anaerobes), normal flora (total streptococci, total actinomyces), putative periodontal pathogens (*P. gingivalis, P. intermedia, B. forsythus, A. actinomycetemcomitans*, or *E. corrodens*), or opportunistic pathogens (*Candida*, enteric bacteria, or *S. aureus*). The only exception was that the total facultative counts were significantly higher ($P = 0.0146$) in the placebo treatment compared to the SDD treatment group in the SRP design at the 6-month sample period. No differences were detected between treatments at any other sample period ($P > 0.3000$).

Within-treatment differences. The means of the colony forming units (CFUs) for total anaerobic counts, facultative counts, and obligate anaerobes obtained at each sample period for each treatment are given in Figures 1 through 3. Statistically significant increases were detected with the paired *t* test in both the total anaerobic counts and the obligate anaerobes present at 3 months relative to baseline for the SDD and placebo treatments in both designs. Significant increases were also detected at 6 months, relative to baseline, for both the SDD and placebo treatments in the non-SRP design. However, when these data were reanalyzed using the Wilcoxon signed rank test to minimize the effects of extreme outliers, statistically significant increases were detected only in the placebo treatment in the non-SRP design for the total anaerobic counts and the obligate anaerobes at 3 and 6 months relative to baseline ($P < 0.02$). Significant increases were noted in the number of facultative counts present at 6 months relative to baseline for the placebo treatment in both the SRP and non-SRP designs, but these increases were not statistically significant when reanalyzed using the Wilcoxon signed rank test. No statistically significant differences were detected within the SDD or placebo treatment groups in the SRP and non-SRP design by either the paired *t* test or Wilcoxon signed rank test in any of the following microbial groups: streptococci, *Actinomyces, P. gingivalis, P. intermedia, B. forsythus, A. actinomycetemcomitans, E. corrodens, Candida*, enterics, or *S. aureus*.

Clinical Parameters Associated With Microbial Sample Sites

Since statistically significant microbial decreases, either between or within treatments, during the 9-month treatment period were associated with motile groups (spirochetes and motile rods) that have been used as indicators of disease activity, the clinical indices obtained for the microbiology sample sites at each sample period were analyzed.

Between-treatment differences. No statistically significant differences were detected between the SDD and placebo treatments in the SRP design for either AL or PD at any sample period. However, in the SRP design, the percentage of BOP sites (Fig. 4) in the SDD group was significantly lower ($P < 0.01$) than the placebo group at all sample periods following baseline. In the non-SRP design, significant gains ($P < 0.01$) in AL were present in the SDD group at 3, 6, and 9

Table 5.

Mean Percentage of Coccoid Forms Relative to Total Microscopic Flora for SDD and Placebo Treatment Groups in SRP and Non-SRP Design

Design	Treatment Group	Baseline	Treatment Phase: 3 Months	6 Months	9 Months	3 Months Post
SRP	SDD	14.87	33.53*	37.04*	41.36*	34.45*
SRP	Placebo	13.11	24.18*	31.31*	37.20*	33.59*
Non-SRP	SDD	14.23	34.76*	39.05*	39.32*	33.33*
Non-SRP	Placebo	13.20	24.04*	30.54*	30.92*	31.53*

* Statistically significant within-treatment differences ($P<0.0001$) relative to baseline.

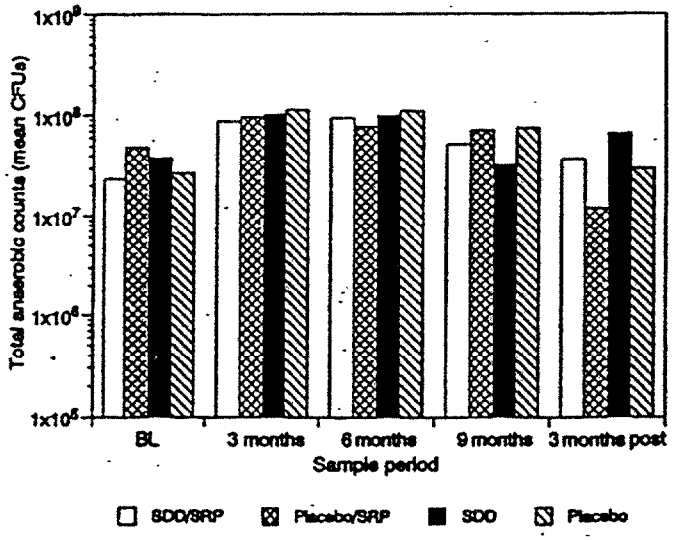

Figure 1.
Total cultivable anaerobic counts (means) obtained for each treatment at each sample period.

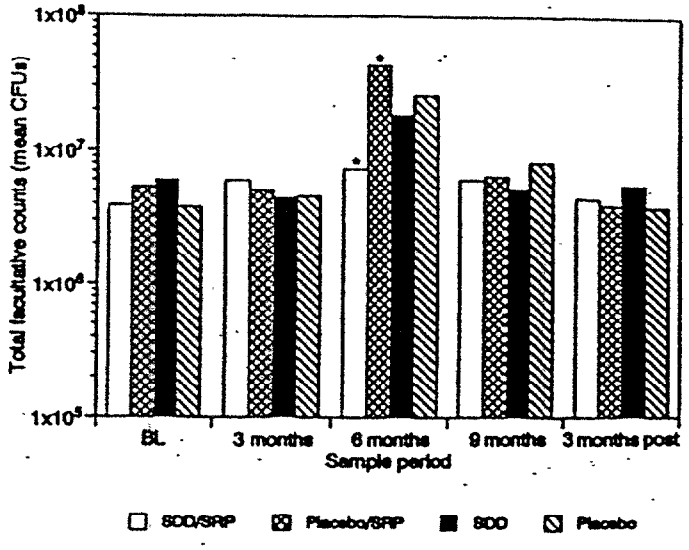

Figure 2.
*Total cultivable facultative counts (means) obtained for each treatment at each sample period (*statistical significance between treatments, $P<0.02$).*

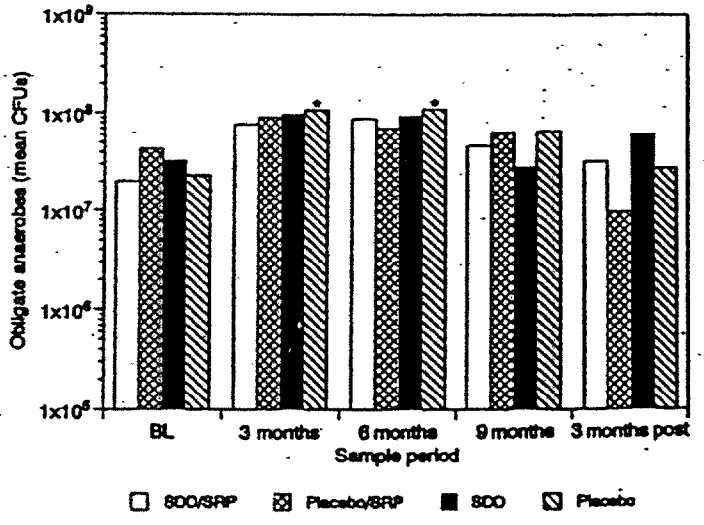

Figure 3.
*Obligate anaerobic counts (means) obtained for each treatment at each sample period (*statistical significance relative to baseline, $P<0.02$).*

months and fewer sites bled on probing ($P<0.005$) at 6 and 9 months. No differences were detected in PD at any sample period.

Within-treatment differences. Statistically significant ($P<0.0001$) increases in AL and decreases in PD were detected at 3, 6, and 9 months, relative to baseline, regardless of treatment or design. No significant differences were detected between either the 3-, 6-, or 9-month measurements relative to each other. Significant decreases in proportion of BOP sites (Fig. 1) were noted at 3, 6, and 9 months, relative to baseline, for the SDD group in the SRP design ($P<0.0005$) and in the non-SRP design ($P<0.01$). Significant decreases in BOP sites were noted in the placebo group in the SRP design at 3 and 6 months ($P<0.001$) relative to baseline but not at 9 months, and in the placebo group in the non-SRP design at 3 months ($P<0.005$) but not at 6 or 9 months.

DISCUSSION

The principal objective of this investigation was to determine whether SDD exerted any detectable effect on the subgingival flora that could be attributed to

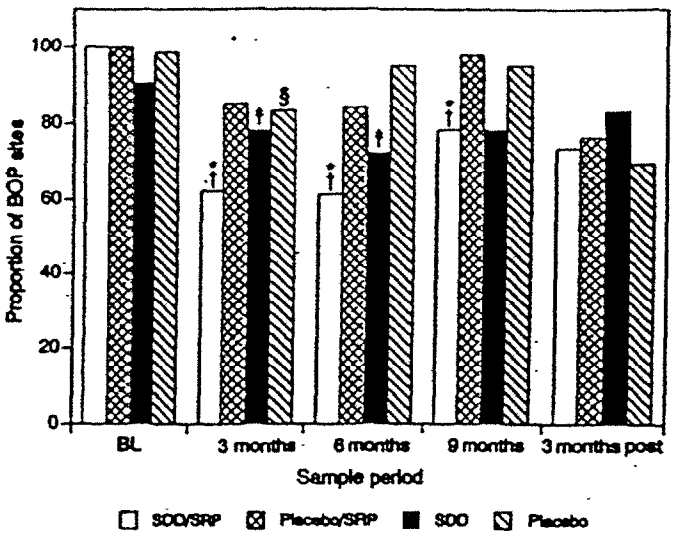

Figure 4.
*Percentage of microbial sample sites bleeding on probing for each treatment at each sample period (*statistical significance between treatments, P <0.01; †relative to baseline, P <0.0005; ‡relative to baseline, P <0.01; §relative to baseline, P <0.005).*

antimicrobial activity. Doxycycline is normally given at a daily dose of 100 mg, following a loading dose of 200 mg, which yields biologically active levels of 8 to 16 μg/ml in the gingival crevicular fluid and around 4 μg/ml in the blood.[11] Studies in human volunteers have demonstrated that 20 mg doxycycline bid yields steady-state serum concentrations of 0.6 to 0.8 μg/ml (unpublished data). This level is considerably below the minimal inhibitory concentration (MIC) determined in vitro for the vast majority of the bacteria isolated from the subgingival flora.[12,13] Since subgingival plaque exists as a biofilm rather than in a planktonic state,[14] even higher drug concentrations are probably necessary for in vivo inhibition. Even so, the possibility exists that levels obtained with SDD might be inhibitory for certain bacteria that are exquisitely sensitive to the tetracyclines. Therefore, in this study, a comprehensive microbial examination of the subgingival flora was conducted by microscopy and culture enumeration in an attempt to detect differences between and within treatments that could be contributed to an antimicrobial effect.

No statistical or microbiological differences in any of the microbial parameters enumerated were detected between SDD and placebo treatments in either the SRP or non-SRP design, with the exception of the spirochetes. In both designs, the small and large spirochetal groups were found to be significantly lower at certain periods in the SDD treatment than in the corresponding placebo group. There are several possible explanations for the suppression of the spirochetes in the SDD groups. One is that the levels of doxycycline obtained in the periodontal pocket are inhibitory for these organisms. Although the large spirochetes have not been cultivated and their sensitivity to the tetracyclines is unknown, it is generally thought that the small spirochetes are relatively sensitive to the tetracyclines, although resistance has been reported.[15] Therefore, it might be argued that the suppression of the spirochetes was due to the antimicrobial activity of doxycycline. However, other bacterial groups are equally sensitive, if not more so. Almost all isolates of *P. gingivalis* are inhibited in vitro by ≤0.25 μg/ml of the tetracyclines.[12,13,16] Neither we nor a number of other investigators have been successful in isolating wild-type strains of this organism with naturally occurring resistance to the tetracyclines. In the study reported here, there were no differences between treatments at either West Virginia University or the University of Florida in the numbers of *P. gingivalis* recovered at any sample period. This tends to argue against the possibility that the decrease in the relative proportion of the spirochetes was due to antimicrobial activity, since corresponding decreases in the numbers or proportions of *P. gingivalis* were not found.

Another possibility that has been advanced is that the decrease in spirochetes was due to the periodontal pocket becoming more aerobic. Since the spirochetes are thought to have a relatively low redox (Eh) requirement for growth,[17] an increase in the Eh of the pocket might favor the growth of more oxygen-sensitive species at the expense of the spirochetes. However, this would most likely occur following mechanical disruption of the structure of the plaque biofilm. If this were the case, one would not expect to find treatment differences between SDD and placebo treatments in the SRP design, since both groups received periodontal scaling prior to the adjunctive treatment regimen.

The most likely explanation for the observed spirochetal differences between treatments is probably related to an improvement in the health of the periodontal pocket. There was significantly less inflammation as determined by the proportion of sites bleeding on probing in both SDD groups relative to placebo. The proportion of bleeding sites was significantly lower in the SDD/SRP group than the placebo group at 3, 6, and 9 months ($P < 0.005$) and in the SDD/non-SRP group at 6 and 9 months ($P < 0.005$). Within-treatment analyses revealed statistically significant improvements for all treatments in AL, PD, and BOP. Concurrently with these improvements in clinical indices, within-treatment analyses detected statistically significant decreases in spirochetes and motile rods with corresponding increases in coccoid forms. Since microscopic motility and bleeding on probing are often useful as indicators of disease activity, it seems reasonable to expect some relationship between the two. Therefore, we think the most logical explanation for the

between-treatment differences in spirochetes is that the microbial sample sites improved in health due to the anti-inflammatory properties of the drug so that fewer nutrients were available to support the growth of spirochetes. It could be argued that the decrease in the spirochetal population was responsible for the improvement in health, with the decrease being due to the antimicrobial activity of the drug. We do not think that this is likely due to the fact that between-treatment differences were not detected in any of the other microbial parameters. If the decrease in the number of sites bleeding on probing was due to an antimicrobial effect, between-treatment differences in microbial parameters should occur prior to the detection of improvements in clinical indices. In this study, the proportion of sites bleeding on probing had decreased prior to the detection of significant between-treatment differences in the proportions of small and large spirochetes. Since the clinical effect was observed before the microbial effect, we think this supports the hypothesis that the between-treatment differences were due to the drug's anti-inflammatory effect rather than to its antimicrobial effect.

In conclusion, no antimicrobial effect could be detected during or following a 9-month treatment regimen with 20 mg doxycycline bid, relative to placebo control, on total bacterial counts, the normal flora, or in either periodontal or opportunistic pathogens. Doxycycline had no detectable antimicrobial effect on 21 different microbial parameters commonly used to evaluate changes in the subgingival microflora.

ACKNOWLEDGMENTS

Mr. Powala is Director of Drug Development and Regulatory Affairs and Ms. Wetzel is a study monitor at CollaGenex Pharmaceuticals, Inc. This study was supported by a grant from CollaGenex Pharmaceuticals, Inc., Newtown, Pennsylvania.

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EXHIBIT F

Long-Term Use of Subantimicrobial Dose Doxycycline Does Not Lead to Changes in Antimicrobial Susceptibility

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Background: Adjunctive subantimicrobial dose doxycycline (SDD) with scaling and root planing leads to improved clinical parameters of adult periodontitis, but has raised questions about potential changes in antibiotic susceptibility of the host microflora. Our four studies assessed whether long-term SDD changes antibiotic susceptibility of the oral microflora in adults with periodontitis.

Methods: In studies 1 and 2, adult patients with periodontitis were randomized to receive SDD 10 mg qd, 20 mg qd, 20 mg bid, or placebo. In study 3, patients were randomized to receive SDD 20 mg bid or placebo. No medication was administered in study 4, a follow-up to study 3. Subgingival plaque samples were collected at baseline (all studies) and at 12, 15 to 18, and 24 months (study 1); 12, 18, and 27 months (study 2); 3, 6, and 9 months (study 3); and 3 months post-study 3 (study 4). Antimicrobial susceptibility of isolated bacteria was assessed by: 1) minimum inhibitory concentration (MIC) levels (studies 1 and 2); 2) cross-resistance to non-tetracycline antibiotics (studies 2 and 3); and 3) the proportion of doxycycline-resistant isolates (studies 3 and 4).

Results: Organism MIC levels remained constant among all treatment groups at 18 and 24 months compared with baseline (study 1). Observed changes in susceptibility at 12 and 18 months for the 20 mg groups were attributed to the limited number of isolates tested (study 1). There were no statistically significant differences in the proportion of doxycycline-resistant isolates among treatment groups (studies 3 and 4), and no evidence of multi-antibiotic resistance (studies 3 and 4) or cross-resistance (studies 2 and 3) at any timepoint.

Conclusion: Long-term SDD does not contribute to changes in antibiotic susceptibility. *J Periodontol 2000;71:1472-1483.*

KEY WORDS

Doxycycline/therapeutic use; drug resistance, microbial; antibiotics/therapeutic use; periodontitis/drug therapy; dose-response relationship, drug; comparison studies.

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Doxycycline has been shown to effectively inhibit collagenase activity in gingival tissues and crevicular fluid, thereby reducing the destruction of collagen in adult periodontitis.[1-4] This doxycycline-induced decrease in collagenase activity is accompanied by a significant improvement in other periodontal disease parameters, such as improvement in periodontal attachment levels and decreasing probing depth.[2-5] A long-term trial evaluating the efficacy of a 9-month regimen of subantimicrobial dose doxycycline (SDD) found that a combined regimen of SDD and scaling and root planing (SRP) resulted in statistically significant improvements in periodontal attachment level and probing depth, when compared to SRP plus placebo.[6,7]

The anticollagenase activity of doxycycline is independent of its antimicrobial activity, as first reported by Golub et al. in 1983[8] and confirmed through subsequent research.[2-4,9] Effective anticollagenase activity is obtained in man at administered doses well below those routinely used for effective antimicrobial treatment. For example, the usual antimicrobial dose of doxycycline is a 200 mg initial dose followed by 100 mg qd, which produces blood levels of 3 μg/ml to 4 μg/ml.[10] When used to suppress collagenase activity, however, the effective dose of doxycycline is 20 mg bid, which produces maximum serum concentrations of 0.79 μg/ml during chronic administration (unpublished data).

Prior studies have failed to detect an antimicrobial effect of doxycycline on subgingival microflora.[10,11] In a study evaluating SDD and placebo treatment with and

without accompanying SRP, Walker et al. found no statistically significant or microbiological differences between or among treatment groups for motile rods, coccoid forms, non-motile rods, fusiforms, and filamentous rods. Only levels of small and large spirochetes were found to be significantly lower in the SDD group than the placebo group.[10] This decrease in spirochetes was not attributed to an antimicrobial effect. Rather, it was explained by an overall improvement in periodontal health due to the anti-inflammatory and anticollagenolytic properties of doxycycline, which alter the microenvironment of the periodontal pocket, to which spirochetes are known to be particularly sensitive.[12]

However, any long-term use of antibiotics raises questions of changes in antibiotic susceptibility.[9,11,13-16] In addition, there are concerns that the suppression of a normal, susceptible microflora could lead to overgrowth of more resistant and potentially pathogenic microorganisms in reservoirs such as the oral cavity.[11]

To confirm the results of previous research, four studies were performed to assess whether the long-term use of SDD changes antibiotic susceptibility of the subgingival microflora in adults with periodontitis. Indications of altered susceptibility were monitored by measuring changes in minimum inhibitory concentrations (MICs) of specific species; examining susceptibility patterns to specific antibiotics (doxycycline, minocycline, tetracycline, amoxicillin, erythromycin, penicillin, ampicillin, cefoxitin, metronidazole, and clindamycin) according to the National Committee for Clinical Laboratory Standards (NCCLS) categories;[17] and by determining alterations in the distribution of susceptibility.

MATERIALS AND METHODS

The methods and treatments used in each of the four studies are summarized in Table 1. In studies 1 and 2, patients were eligible for study participation if they were between 35 and 75 years of age; in studies 3 and 4, patients were included if they were between 30 and 75 years of age. All patients had a clinical diagnosis of periodontitis. Periodontitis was defined as both clinical attachment levels ≥5 mm and ≤9 mm and probing depths ≥5 mm and ≤9 mm in at least two subgingival tooth sites within the full mouth (studies 1 and 2) or in 2 tooth sites in each of 2 quadrants (i.e., 4 sites) (studies 3 and 4).

Patients were excluded if they had received any antibiotics within 6 weeks of the baseline visit or if they required chronic antibiotic treatment (i.e., more than 2 weeks) or prophylactic antibiotics for routine dental therapy. Women who were pregnant or lactating were also excluded, as were patients diagnosed with diabetes mellitus, a serious medical illness (e.g., kidney or liver disease), or a systemic infection. Additionally, patients with known hypersensitivity to tetracyclines or who were taking significant concomitant

Table 1.

Summary of Studies

Study	N*	Method	Target Organisms	Treatment Groups	Assessment Periods
1	30	Randomized, double-blind (12 months), placebo-controlled, parallel study	*Actinomyces* spp. *Fusobacterium* spp.	10 mg qd doxy 20 mg qd doxy 20 mg bid doxy Placebo	Baseline, 3 months, 6 months, 9 months, 12 months
2	40	Randomized, 12-month blinded, 6 months open-label, 9 months no treatment	*Actinomyces* spp. *Fusobacterium* spp.	10 mg qd doxy 20 mg qd doxy 20 mg bid doxy Placebo	Baseline, 3 months, 6 months, 9 months, 12 months
3	171	Randomized, placebo-controlled, double-blind, parallel group. In each patient, two quadrants treated with SRP, two without SRP.	Predominant oral flora	20 mg bid doxy Placebo	Baseline, 3 months, 6 months, 9 months, 12 months
4	146‡	Follow-up of study 3	Predominant oral flora	No further administration of study drug	3 months (active treatment), 6 months, 9 months, 12 months
Totals	251‡				

* Total number of patients.
† Doxy = doxycycline.
‡ One hundred forty-six (146) patients in study 4 were not unique patients but a subpopulation of study 3 and were not counted in the total.

therapy were also excluded from the studies. Clinical and microbial data were collected at the University of Florida, Gainesville, and West Virginia University, Morgantown.

Study 1 Methods

Study 1 was a double-blind, placebo-controlled, parallel study with an open-label extension. Patients were administered SDD (20 mg qd, 20 mg bid, or 10 mg qd) or placebo for 12 months, after which they could opt to enter an open-label phase lasting 3 to 6 months. Patients continued their dosing regimen during the open-label phase. Patients received a supragingival prophylaxis at baseline, at 6 and 12 months, and at study exit (15 to 18 months). Additionally, patients were asked to return for further analysis 6 months after treatment ended (21 to 24 months post-baseline).

Subgingival plaque samples were collected with sterile endodontic paper points at baseline and at 12 months, 15 to 18 months (on cessation of treatment), and 6 months post-treatment. Each sample was assessed for total cultivable anaerobic bacteria, total *Actinomyces* spp isolates, and total *Fusobacterium* spp isolates to determine the effect of the SDD or placebo regimen on microbial flora. *Actinomyces* spp and *Fusobacterium* spp were selected as representative Gram-positive and Gram-negative isolates known to be encountered in a patient population with moderate to severe periodontal disease. Previous research has demonstrated that these are appropriate marker organisms for evaluating the development of resistance when using SDD.[18]

Representative *Actinomyces* and *Fusobacterium* isolates were obtained from each sample to test susceptibility to doxycycline (i.e., minimum inhibitory concentration [MIC] values). Trypticase-soy agar supplemented with 5% whole defibrinated sheep blood, 0.005% hemin, and 0.0005% menadione was used as a non-selective medium for total anaerobic counts. Susceptibility testing was performed by an agar dilution method.[17,19,20] To assess clinical efficacy, clinical attachment levels and probing depths were measured at 6 sites around each tooth in the whole mouth by manual probing at baseline, 6 months, and 12 months.

ANOVA was used to determine whether statistically significant differences in total organisms isolated existed among sample periods. Fisher's PLSD analysis was used to determine whether differences in total organisms isolated existed between two treatments at each sample period.

Study 2 Methods

Study 2 was similar in design to study 1. Patients were administered SDD or placebo for 12 months, after which they entered an open-label phase (i.e., treatment was not blinded) lasting 3 to 6 months. Patients continued their dosing regimen during the open-label phase and received a supragingival prophylaxis at baseline, at 6 and 12 months, and at the cessation of treatment. Patients returned up to 9 months after cessation of treatment for further analysis (i.e., up to 27 months post-baseline).

Subgingival plaque samples were collected by curet at baseline, 12 months (at the end of blinded treatment), 18 months (after the 6-month open-label phase), and up to 27 months (after 9 months without treatment). As in study 1, *Actinomyces* spp and *Fusobacterium* spp were isolated using a non-selective complex medium[21,22] as representative taxa common in the periodontal microflora.[18] The isolates were tested for resistance (i.e., MIC values) to tetracycline, erythromycin, penicillin, ampicillin, cefoxitin, and metronidazole using antibiotic-impregnated strips[§] (used by West Virginia University) or agar dilution (used by the University of Florida).[19,20,23] To assess efficacy, clinical attachment levels and probing depths were measured at 6 sites around each tooth by manual probing at baseline, 6 months, and 12 months.

Study 3 Methods

Study 3 was a multi-center, randomized, parallel-group, placebo-controlled study, and study 4 was a 3-month follow-up of study 3. In study 3, all patients were treated in two qualifying quadrants with SRP at baseline. To be considered a qualifying quadrant, two tooth sites in each quadrant had to have both clinical attachment level and probing depth ≥5 mm and ≤9 mm, as measured by manual probing. SRP was performed by the same therapist at each study center and lasted for up to 1 hour per quadrant. Both ultrasonic and universal or area-specific curets were permitted, as was local anesthesia. After SRP was performed, patients in study 3 were randomly assigned to receive either SDD 20 mg bid or placebo for 9 months.

In each patient, two sites with probing depths >5 mm from the SRP quadrants and two sites with probing depths >5 mm from non-SRP quadrants were selected for microbiological sampling. Subgingival plaque samples were taken from these sites using sterile endodontic paper points at baseline and after 3, 6, and 9 months of treatment. The same sites were sampled throughout the study. Samples from the SRP sites were pooled by subject and then processed; the same was done for non-SRP sites. Samples were examined by direct microscopy, culture on selective and nonselective media, and predominant cultivable technique. Samples were also tested for susceptibility to doxycycline, minocycline, tetracycline, amoxicillin, erythromycin, and clindamycin by either agar dilution method[19,20,23] or antibiotic-impregnated strips.[§] To assess efficacy as an adjunct to SRP, clinical attachment levels and probing depths were measured by

§ Etest, AB Biodisk, Solna, Sweden.

manual probing at each of 6 tooth sites around each tooth in the qualifying quadrants at baseline and at 3, 6, and 9 months. To check for rapid attachment loss requiring additional intervention, similar measurements were also made around the teeth in the non-qualifying quadrants.

Statistical Methods

The null hypothesis (i.e., the distribution of the strains identified as resistant to 4 μg of doxycycline per milliliter across 3 resistance categories did not differ between placebo and SDD treatment groups) was tested separately for plaque samples from SRP and non-SRP quadrants. The results of the 2 analyses were similar. Separate analyses were carried out for each visit of the study. Data from the 2 centers were pooled, and the center was treated as a factor in the analyses. Of particular interest was the alternative hypothesis that at some study visits, strains isolated from plaque samples from SDD-treated patients may have exhibited greater resistance to doxycycline than those taken from patients treated with placebo. This tendency, if present, would result in a greater proportion of resistant strains falling in the higher resistance categories for SDD-treated patients. Therefore, analyses focused on the degree of resistance observed among resistant strains and differences in the degree of resistance over time and between treatment groups, rather than the raw number or proportion of resistant strains isolated.

Two types of analyses were performed: analyses based on frequency distributions within and between patients, and non-parametric analyses based on scores derived from within-patient frequency distributions. The number of resistant strains isolated from a single patient's plaque sample could vary from 0 to 3; however, plaque samples with no resistant isolates were dropped from the analysis. As mentioned above, it is important to note that these analyses focus on shifts in the degree of resistance among resistant strains rather than changes in the number or proportion of resistant strains.

Frequency distribution analysis. Because multiple strains could be isolated at a given visit within a patient, but the basic unit of analysis was considered to be the patient rather than the microorganism, standard chi-square or Cochran-Mantel-Haenszel analyses could not be used without an adjustment to the degrees of freedom. Frequency distribution analyses were therefore performed by first normalizing the data. Organism counts for each patient visit were transformed into proportions of strains within each resistance category for that patient visit. Normalized individual patient/visit cell counts therefore always summed to unity across the 3 resistance categories, yielding correct overall degrees of freedom. Cochran-Mantel-Haenszel tests were performed on the normalized data, treating [illegible] ters as strata and treating the resistance category as an ordinal parameter with equal spacing between the categories. Scores of 2, 3, and 4 were used for each resistance category based on $\log^2$ of the value of the category in micrograms per milliliter. However, the results of the analysis would not differ using any arbitrary, equally spaced set of numbers for category values.

Non-parametric (ranked) analysis of variance. A single resistance score for each patient visit was created by multiplying the above category scores (2, 3, or 4) by the proportion of resistant strains in each category, then adding the results together. The resulting scores were transformed into ranks, and the ranks were analyzed using an analysis of variance model with factors for treatment group, center, and the interaction of treatment-by-center. This non-parametric procedure produces results that are invariant with respect to the choice of category scores, given that scores are equally spaced. As with the frequency of distribution analysis, the individual patient was taken as the unit of analysis. All analyses reflect the assumption that increased doxycycline resistance would be reflected in a tendency for more strains to fall within the higher resistance categories within a patient/visit. P values ≤ 0.05 were considered statistically significant. Post-hoc power analyses suggested that actual differences greater than 15% would typically have been detected with a probability of 0.80. All statistical tests were 2-tailed. All statistical analyses and data manipulations were performed using statistical software.[1]

Study 4 Methods

No medication was administered during study 4. Subgingival plaque samples were collected from the same sites as those used in study 3 (two sites from the SRP quadrants and two from the non-SRP quadrants) at baseline and at 3 months for analysis of oral flora and antibiotic susceptibility. The two samples from the two SRP quadrants were pooled by subject and then processed; the same was done for non-SRP sites. Microbial samples were examined by either darkfield or phase-contrast microscopy, culture on selective and non-selective media, and predominant cultivable technique. To assess efficacy, clinical attachment levels and probing depths were measured by manual probing.

To determine whether statistically significant differences existed between the SDD and placebo treatment groups, the microbial parameters associated with microscopic evaluation and culture enumeration were analyzed using the unpaired t test and the Mann-Whitney test, a non-parametric version of the two sample, unpaired t test.

[1] SAS 6.12, SAS Institute, Inc., Cary, NC.

RESULTS

Study 1 Results

A total of 40 subjects were included in study 1, 10 in each of 4 treatment groups. All subjects provided microbiological samples at baseline. At 12 months, 29 subjects were sampled (8 in the SDD 20 mg qd treatment group; 7 in the SDD 20 mg bid treatment group; 7 in the SDD 10 mg qd treatment group; and 7 in the placebo group). At 15 to 18 months (study exit), 33 subjects were sampled (9 in the SDD 20 mg qd group; 10 in the SDD 20 mg bid group; 8 in the SDD 10 mg qd group; and 6 in the placebo group). Six months post-treatment, samples were obtained from 24 subjects (7 in the SDD 20 mg qd group; 7 in the SDD 20 mg bid group; 4 in the SDD 10 mg qd group; and 6 in the placebo group).

Effect on microbial flora. Treatment effect on microbial flora was assessed in study 1 by measuring total cultivable anaerobic bacteria, total *Actinomyces* spp isolates, and total *Fusobacterium* spp isolates. Assessments were made for each treatment at each sample timepoint based on the number of cultivable bacterial counts obtained for each target genus.

No statistically significant differences in total anaerobic bacteria, *Actinomyces* spp, and *Fusobacterium* spp isolates were found in study 1 among sample periods (Figs. 1A through 1C; Table 2) or between treatments at baseline, 12 months, or 15 to 18 months ($P > 0.12$). Statistically significant differences were detected, however, in three instances at the 6-month post-treatment sample period (21 to 24 months post-baseline; $P \leq 0.05$). The difference in mean total anaerobic bacteria was higher ($P \leq 0.05$) in the 10 mg qd treatment group compared with the 20 mg qd treatment group. Differences in total *Actinomyces* spp isolates were significant between the 10 mg qd treatment group and the 20 mg qd treatment group, as well as between the 10 mg qd and placebo treatment groups. It was not feasible to conduct a longitudinal analysis of these differences because an inadequate number of the same patients were present in each treatment cell at each sample period to use a repeated measure ANOVA.

Susceptibility. The MIC was evaluated, as were the minimum inhibitory concentrations needed to inhibit growth of 50% (MIC_{50}) or 90% (MIC_{90}) of the target organisms. MIC_{50} or MIC_{90} summaries were determined for comparative purposes. At the 12-month assessment and at 15 to 18 months (study exit), no changes were apparent in the doxycycline MIC_{50} (Fig. 2A) or MIC_{90} (data not shown) for the *Actinomyces* spp isolates in the 10 mg qd treatment group compared with the placebo. At 12 months post-baseline, there was an apparent change in susceptibility to doxycycline in the *Actinomyces* spp isolates in the 20 mg qd and 20 mg bid treatment groups compared with base-

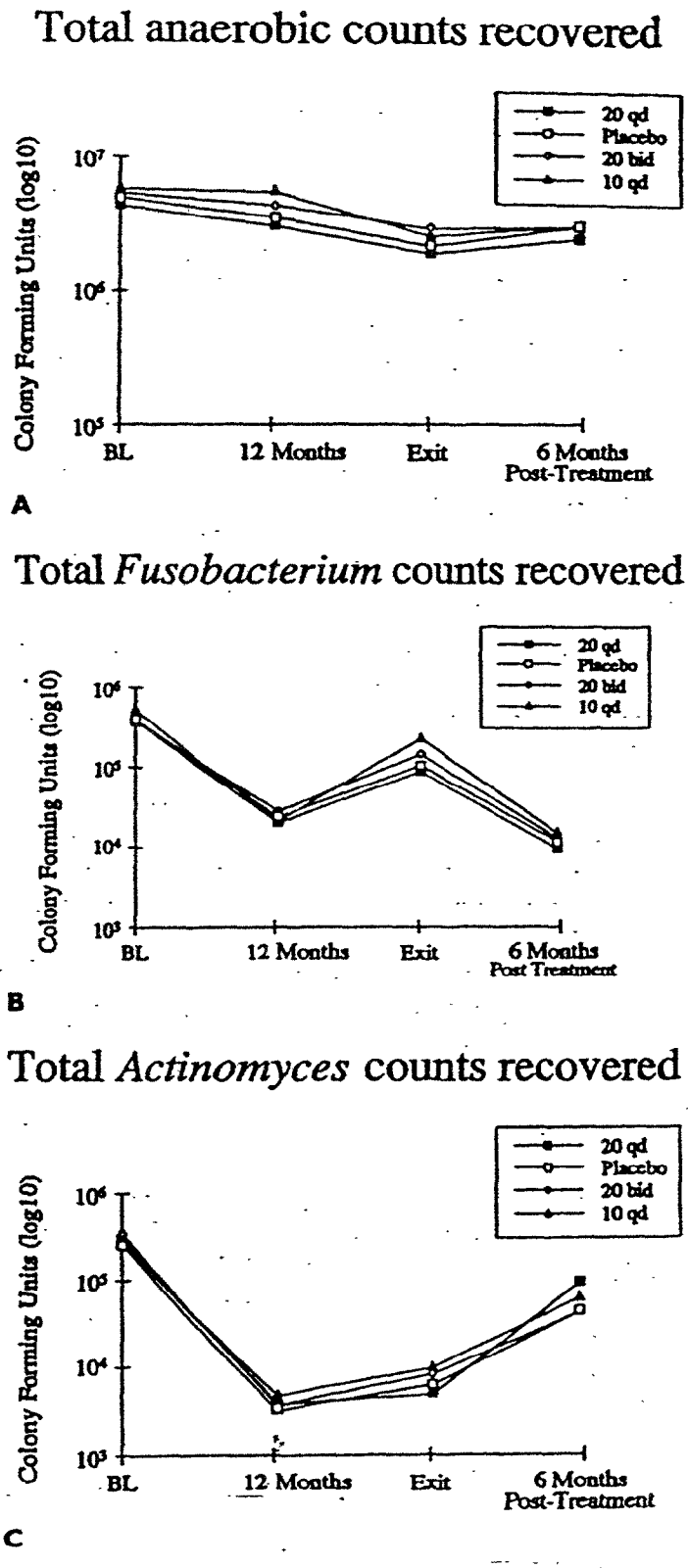

Figure 1.
A. Total cultivable anaerobic counts recovered for each treatment at each sample period. B. Total cultivable Fusobacterium spp counts recovered for each treatment at each sample period. C. Total cultivable Actinomyces spp counts recovered for each treatment at each sample period.

Table 2.

ANOVA Analysis of Differences in Total Anaerobic Bacteria, *Actinomyces* spp, and *Fusobacterium* spp Counts Among Treatment Groups (study 1)

	Sample Period			
Colony Count (LC) Bacterial Group	Baseline (*P* Value)	12 Months (*P* Value)	15 to 18 Months (*P* Value)	6 Months Post-Treatment (*P* Value)
Total anaerobic bacteria	0.8584	0.8775	0.8018	0.1918
Actinomyces isolates	0.4870	0.5007	0.5208	0.5380
Fusobacterium isolates	0.3216	0.4274	0.5427	0.0981

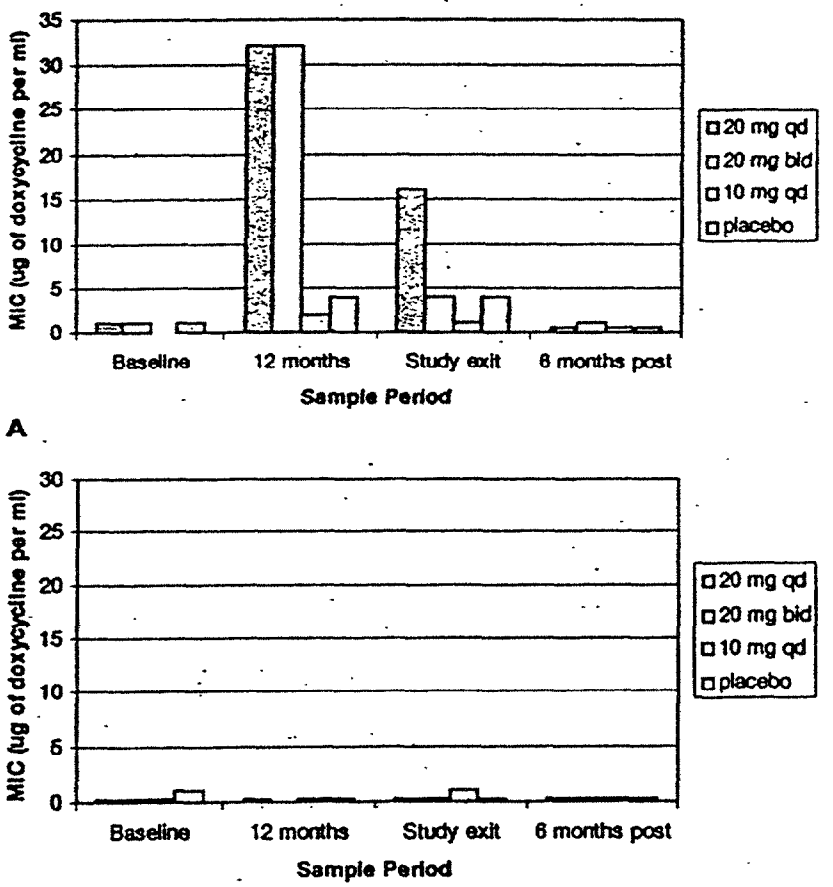

Figure 2.
A. Doxycycline MIC_{50} values for Actinomyces spp isolates for each sample period by treatment group. B. Doxycycline MIC_{50} values for Fusobacterium spp isolates for each sample period by treatment group.

line. However, 15 to 18 months post-baseline, the MIC_{50} and MIC_{90} values for the 20 mg bid treatment group were similar to the placebo, although the MIC_{50} and MIC_{90} values for the 20 mg qd treatment group remained elevated compared with other treatments. At assessments taken 6 months after treatment ended (21 to 24 months post-baseline), no differences in MIC_{50} or MIC_{90} values were found for any of the four treatment groups relative to each other or to baseline for *Actinomyces* spp isolates (Table 3).

The MIC_{50} values for *Fusobacterium* spp isolates were essentially identical for all treatment groups at all sample periods (Fig. 2B). Increases were noted in the doxycycline MIC_{90} of *Fusobacterium* spp isolates in all treatment groups, including placebo, at 15 to 18 months compared with baseline (data not shown). There was no difference, however, among the treatment groups in the doxycycline MIC_{90} at the 12-month assessment. At assessments taken 6 months after treatment ended (21 to 24 months post-baseline), no differences in MIC_{50} or MIC_{90} values were found for any of the four treatment groups relative to each other or to baseline for *Fusobacterium* spp isolates.

Study 2 Results

Patterns and cross-resistance. Among the *Actinomyces* spp and *Fusobacterium* spp isolates collected in study 2, there were no changes in NCCLS antibiotic patterns (Susceptible [i.e., MIC ≤4 µg/ml], Intermediate [i.e., MIC 5 to 15 µg/ml], or Resistant [i.e., MIC ≥16 µg/ml]) nor significant changes in antibiotic susceptibility to the six antibiotics tested (tetracycline, erythromycin, penicillin, ampicillin, cefoxitin, and metronidazole) in any of the sample periods for any of the treatment groups. The MIC_{50} for each antibiotic tested was well below the NCCLS cut-off levels that determine antimicrobial resistance for each of the sample periods (Table 4). Antibiotic profiles and cumulative MIC_{50} remained stable regardless of doxycycline dosage, indicating the absence of multiple antibiotic resistance.

Studies 3 and 4 Results

Distribution of resistance. At baseline and at 3, 6, and 9 months, five taxa accounted for 57% to 80% of doxycycline-resistant isolates (*Bacteroides coagulans, Campylobacter concisus, Eusobacterium* spp, *Prevotella* spp, and *Streptococcus* spp). Four of these taxa accounted for 60% to 80% of doxycycline-resistant isolates at 12 months (*C. concisus, Fusobacterium* spp, *Prevotella* spp, and *Streptococcus* spp). These resistant taxa were consistent

...ble 3.

Doxycycline Susceptibilities for Representative Gram-Positive (*Actinomyces*) and Gram-Negative (*Fusobacterium*) Bacteria

Treatment	N Subjects	Taxa	N Strains	MIC Range	MIC_{50}
Baseline					
20 mg qd	10	*Actinomyces*	6	≤0.25 –	
		Fusobacterium		≤0.25 –	
20 mg bid		*Actinomyces*		≤0.25	
		Fusobacterium			
10 mg qd	10	*Actinomyces*	8	0.5	
		Fusobacterium		≤0.25	
Placebo		*Actinomyces*		≤0.25	
		Fusobacterium	10	≤0.25	
12 months					
20 mg qd	8	*Actinomyces*	6	1 – >32	32
		Fusobacterium	5	≤0.25 – 4	≤0.25
20 mg bid		*Actinomyces*	3	0.5 – >32	32
		Fusobacterium	0	N/A	N/A
10 mg qd		*Actinomyces*	7	2.0 – 8	
		Fusobacterium	5	≤0.25 – 8	≤0.25
Placebo		*Actinomyces*	2	4 – 8	4
		Fusobacterium	2	≤0.25 – 32	≤0.25
15–18 months					
20 mg qd	9	*Actinomyces*	5	≤0.25 – >32	16
		Fusobacterium	2	≤0.25 – 32	≤0.25
20 mg bid	10	*Actinomyces*	6	0.5 – >32	4
		Fusobacterium	4	≤0.25 – >32	≤0.25
10 mg qd	8	*Actinomyces*	4	1.0 – >32	1
		Fusobacterium	4	≤0.25 – >32	1
Placebo	6	*Actinomyces*	3	0.5 – 8	4
		Fusobacterium	2	≤0.25 – >32	≤0.25
Post-treatment					
20 mg qd		*Actinomyces*		≤0.25	
		Fusobacterium		≤0.25	
20 mg bid		*Actinomyces*		≤0.25	
		Fusobacterium		≤0.25	
10 mg qd		*Actinomyces*		≤0.25	
		Fusobacterium		≤0.25	
Placebo	6	*Actinomyces*	28	≤0.25 – 16	0.5
		Fusobacterium	4	≤0.25	≤0.25

not applicable.

across treatment groups (active versus placebo) and intervention groups (SRP versus non-SRP). The proportions of resistant taxa were similar at each timepoint (Table 5).

In assessing the distribution of doxycycline resistance, there were no statistically significant differences between treatment groups. The distribution of doxycycline-resistant isolates was consistent across NCCLS resistance categories at all sample periods after the baseline assessment. This finding was consistent between SRP and non-SRP intervention categories (Figs. 3A through 3C). Coefficients of variance for these analyses ranged from 27% to 55%, with the greatest variance observed at baseline.

No evidence of multi-antibiotic resistance was found, as defined by resistance to two or more unrelated non-tetracycline antibiotics. The MIC values of the doxycycline-resistant isolates were evaluated, and statistical testing for correlation was performed for each treatment at each sample period to determine if a correlation existed between resistance to doxycycline and resistance to each of the other antibiotics (minocycline, tetracycline, amoxicillin, erythromycin, and clindamycin). A strong correlation was found between resistance to doxycycline and minocycline (0.600 – 0.950), with a weaker correlation between resistance to doxycycline and tetracycline (0.200 – 0.800) for all treatments at all sample periods. Additionally, a relatively strong correlation was found between resistance to erythromycin and clindamycin (0.450 – 0.900) for all treatments at all sample periods. No correlations were found between doxycycline resistance and resistance to non-tetracycline antibiotics (erythromycin, clindamycin, or amoxicillin) at any sample period.

DISCUSSION

The anticollagenase activity of doxycycline is evident at doses below those routinely used for antimicrobial treatment.[2-4,8,9] To suppress collagenase activity, the effective dose of doxycycline is 20 mg bid, compared with the usual antimicrobial dose of 200 mg for the initial dose followed by 100 mg qd.

Long-term antibiotic use, however, raises questions regarding changes in antibiotic susceptibility. Taken together, the four studies reported here (three separate clinical trials and one 3-month follow-up study) evaluated whether long-term use of SDD resulted in altered antimicrobial susceptibility in adults with periodontitis, as measured by MIC levels (study 1), by altered susceptibility to antibiotics other than tetracyclines (study 2), and by the proportion of antibiotic-resistant isolates obtained (studies 3 and 4).

Table 4.

Cumulative Antibiotic Data for Six Antibiotics at Each Sample Period by Treatment Group: MIC_{50} and Range (study 2*)

Sample Period and Treatment	N Patients	N Isolates by Taxa† (n)	MIC_{50} (Range) (μg/ml)‡ Antibiotic: Tetracycline	Erythromycin	Penicillin	Ampicillin	Cefoxitin	Metronidazole
Baseline								
Placebo	8	A (6)	0.125 (<0.016-0.250)	<0.016 (<0.016-0.064)	0.016 (<0.016-0.190)	0.016 (<0.016-0.032)	<0.016 (<0.016-0.190)	>32 (>32)
		F (2)	0.380 (0.380-0.750)	4.0 (4.0-8.0)	0.016 (0.016->256)	0.016 (0.016->256)	0.250 (0.250->256)	<0.002 (<0.002-0.003)
10 mg qd	7	A (6)	0.190 (<0.016-0.750)	0.023 (<0.016-0.047)	0.032 (<0.016-0.047)	0.023 (<0.016-0.047)	0.125 (<0.016-0.094)	>32 (>32)
		F (1)	<0.016 (<0.016)	<0.016 (<0.016)	>256 (>256)	<0.016 (<0.016)	<0.016 (<0.016)	<0.002 (<0.002)
20 mg qd	10	A (8)	0.190 (<0.016-0.500)	0.023 (<0.016-0.047)	0.032 (<0.016-0.094)	0.023 (<0.016-0.047)	0.125 (<0.016-1.0)	>32 (8->32)
		F (1)§	0.125 (0.125)	96 (96)	>256 (>256)	>256 (>256)	>256 (>256)	<0.002 (<0.002)
20 mg bid	6	A (5)	0.250 (<0.016-1.000)	0.032 (<0.016-0.094)	0.023 (<0.016-0.047)	0.016 (<0.016-0.125)	0.016 (<0.016-6.0)	>32 (>32)
		F (1)	0.500 (0.500)	0.500 (0.500)	0.023 (0.023)	0.032 (0.032)	0.125 (0.125)	0.004 (0.004)
12 months								
Placebo	8	A (6)‖	0.380 (<0.016-2.0)	<0.016 (<0.016-0.094)	<0.016 (<0.016-0.064)	<0.016 (<0.016-0.094)	<0.016 (<0.016-0.125)	>32 (>32)
		F (2)	<0.016 (<0.016-0.500)	32 (32->256)	<0.016 (<0.016-0.047)	<0.016 (<0.016-0.094)	<0.016 (<0.016-0.032)	0.050 (0.050-1.0)
10 mg qd	7	A (7)	0.500 (<0.016-2.0)	0.032 (<0.016-0.064)	0.023 (<0.016-0.047)	<0.016 (<0.016-0.032)	0.023 (<0.016-0.190)	>32 (>32)
		F (0)	—	—	—	—	—	—
20 mg qd	9	A (7)	1.000 (<0.016-1.50)	0.047 (<0.016-0.125)	0.032 (<0.016-0.094)	0.032 (<0.016-0.190)	0.032 (<0.016-8.0)	>32 (>32)
		F (2)	0.380 (0.380-1.000)	3.0 (3.0-4.0)	<0.016 (<0.016)	<0.016 (<0.016)	0.064 (0.064-0.094)	0.064 (0.064-0.094)
20 mg bid	4	A (4)	0.500 (0.380-1.50)	0.047 (0.023-0.094)	0.032 (0.032-0.125)	0.032 (0.032-0.064)	0.125 (0.094-0.250)	>32 (>32)
		F (0)	—	—	—	—	—	—

* Representative data from a single center (West Virginia University, Morgantown) only.
† A=*Actinomyces*; F=*Fusobacterium*.
‡ NCCLS breakpoints for resistance: tetracycline=≥16 μg/ml; erythromycin=≥16 μg/ml; penicillin=≥8 μg/ml; ampicillin=≥ 8 μg/ml; cefoxitin=≥64 μg/ml; metronidazole=≥32 μg/ml.
§ One isolate did not provide results for the antibiotic strip (all antibiotics).
‖ One isolate did not provide results for the antibiotic strip (metronidazole).

Table 4. (continued)

Cumulative Antibiotic Data for Six Antibiotics at Each Sample Period by Treatment Group: MIC_{50} and Range (study 2*)

Sample Period and Treatment	N Patients	N Isolates by Taxa† (n)	MIC_{50} (Range) (μg/ml)† Antibiotic: Tetracycline	Erythromycin	Penicillin	Ampicillin	Cefoxitin	Metronidazole
18 months								
Placebo	5	A (5)	1.5 (0.50-16.0)	0.023 (<0.016-16)	1	0.25 (0.064-0.75)	1.5 (0.12->256)	>32 (>32)
		F (1)	0.25 (0.25)	<0.016 (<0.016)	1	0.38 (0.38)	0.190 (0.190)	>32 (>32)
10 mg qd	3	A (3)	0.50 (<0.016-1.2)	0.016 (<0.016-0.50)	1	0.125 (0.047-0.190)	<0.016 (<0.016-0.250)	>32 (>32)
		F (0)	—	—	1	—	—	—
20 mg qd	7	A (6)	0.50 (0.38-1.5)	0.032 (<0.016-0.094)	1	0.250 (0.032-0.750)	0.125 (<0.016-2.0)	>32 (>32)
		F (1)#	—	<0.016 (<0.016)	1	—	—	—
20 mg bid	3	A (3)#	2.0 (2.0-24)	0.032 (0.032-0.047)	1	0.250 (0.250-0.380)	0.190 (0.190-0.190)	>32 (>32)
		F (0)	—	—	1	—	—	—
Post-treatment								
Placebo	8	A (8)	0.125 (0.032-0.750)	0.032 (0.016-0.750)	0.032 (<0.002-0.064)	0.032 (<0.016-0.500)	0.125 (<0.016-0.380)	>32 (>32)
	0	F (0)	—	—	—	—	—	—
10 mg qd	3	A (3)	1.00 (0.25-24.0)	0.094 (0.064-0.125)	0.064 (0.032-0.064)	0.094 (0.064-0.190)	1.5 (0.380-2.0)	>32 (>32)
	2	F (2)	0.50 (0.50-0.380)	3.0 (3.0->256)	<0.002 (<0.002)	<0.016 (<0.016-0.032)	0.023 (0.023-0.032)	0.064 (0.064)
20 mg qd	7	A (9)**	0.075 (0.032-0.500)	0.094 (0.032->256)	0.032 (<0.002-0.094)	0.032 (<0.016-0.190)	0.047 (0.016-1.50)	>32 (>32)
	1	F (1)	0.380 (0.380)	8.0 (8.0)	<0.002 (<0.002)	<0.016 (<0.016)	<0.016 (<0.016)	0.064 (0.064)
20 mg bid	3	A (4)††	0.125 (<0.016-0.170)	0.016 (0.016-0.094)	<0.002 (<0.002-0.094)	<0.016 (<0.016-0.250)	<0.016 (<0.016-6.0)	>32 (>32)
	1	F (1)	0.380 (0.380)	16 (16)	<0.002 (<0.002)	<0.016 (<0.016)	<0.016 (<0.016)	0.032 (0.032)

1 Antibiotic-impregnated strips not available from inventory. For comparative results, see ampicillin.
One isolate did not provide results for the antibiotic strip (tetracycline, ampicillin, cefoxitin, and metronidazole).
** Multiple isolates from same patient (all antibiotics).
†† One isolate did not provide results for the antibiotic strip (erythromycin). Multiple isolates recovered from same patient (metronidazole).

Table 5.

Percentage of Predominant Bacterial Taxa Resistant to ≥4 μg/ml of Doxycycline

	SDD				Placebo			
Bacterial Taxa	Baseline	3 Months	6 Months	9 Months	Baseline	3 Months	6 Months	9 Months
SRE								
Bacteroides ureolyticus	18.67	4.71	4.26	0.00	10.13	2.25	2.82	0.00
Campylobacter concisus	6.67	3.53	14.89	5.33	16.46	5.62	15.49	3.80
Fusobacterium spp	6.67	5.88	18.09	14.67	8.86	14.61	18.31	12.66
Prevotella spp	4.00	15.29	15.96	17.33	7.59	11.24	18.31	6.33
Streptococcus spp	38.67	28.24	25.53	42.67	27.85	31.46	23.94	49.37
Total	74.68	57.65	78.73	80.00	70.89	65.18	78.87	72.16
Non-SRE								
Bacteroides ureolyticus	12.99	2.60	5.26	0.00	11.90	2.22	4.40	1.19
Campylobacter concisus	6.49	2.60	17.89	6.49	16.67	10.00	6.59	4.76
Fusobacterium spp	10.39	16.88	17.89	15.58	9.52	15.56	12.09	10.71
Prevotella spp	9.09	18.18	13.68	9.09	3.57	17.78	12.09	7.14
Streptococcus spp	19.48	15.58	21.05	41.56	33.33	25.56	30.77	41.67
Total	58.44	55.84	75.77	72.72	74.99	71.12	65.94	65.47

In study 1, no changes were detected in MIC_{50} or MIC_{90} values except at 12 months, when there was an apparent change in susceptibility to doxycycline in the *Actinomyces* spp isolates in the 20 mg qd and 20 mg bid treatment groups compared with baseline. The MIC_{50} values for *Fusobacterium* spp isolates were essentially identical for all treatment groups at all sample periods. Six months after treatment ended (21 to 24 months post-baseline), no differences in MIC_{50} values were found for any of the four treatment groups relative to each other or to baseline for *Actinomyces spp* or *Fusobacterium spp* isolates.

Accurate determinations of MIC values require the testing of a sufficient number of strains and patients to represent the population as a whole. If only a few strains are available for testing, a single outlier with a high MIC value can give a misleading impression that a significant decrease in susceptibility has developed over the course of treatment. This appears to be the case in both the 12-month and 15- to 18-month samples assessed in study 1, where a limited number of patients were available for sampling at these timepoints and few carried the target bacteria. Thus, the MIC values obtained for *Actinomyces* spp isolates for the 20 mg treatment groups at 12 months give an initial impression of reduced susceptibility to doxycycline.

However, the resolution of these differences by 15 to 18 months and at 21 to 24 months suggests that the 12-month data were transient and a function of normal microbial variation amplified by the small sample size. No difference was observed at 6 months, at which time more isolates were available for testing. This is also the likely explanation for the apparent increase in resistance among the *Fusobacterium* spp isolates at 15 to 18 months in all treatment groups, including the placebo group. This hypothesis is reinforced by the low MIC values obtained for the target microorganisms for all four treatment groups at the 6-month post-treatment sample. Total bacterial counts recovered in study 1 revealed no evidence for suppression of the subgingival microflora.

As indicated in study 2, SDD was not associated with development of resistance in the marker bacteria (*Actinomyces* spp isolates) independent of the levels at which doxycycline was administered. There were only limited changes in the Susceptible category for the *Actinomyces* spp isolates using NCCLS guidelines; of the 245 *Actinomyces* spp isolates recovered and assayed by the antibiotic-impregnated strips and agar dilution method in studies 1 and 2, only 8 had MIC levels ≥16 μg/ml, indicating tetracycline resistance. Two of these resistant isolates were recovered from

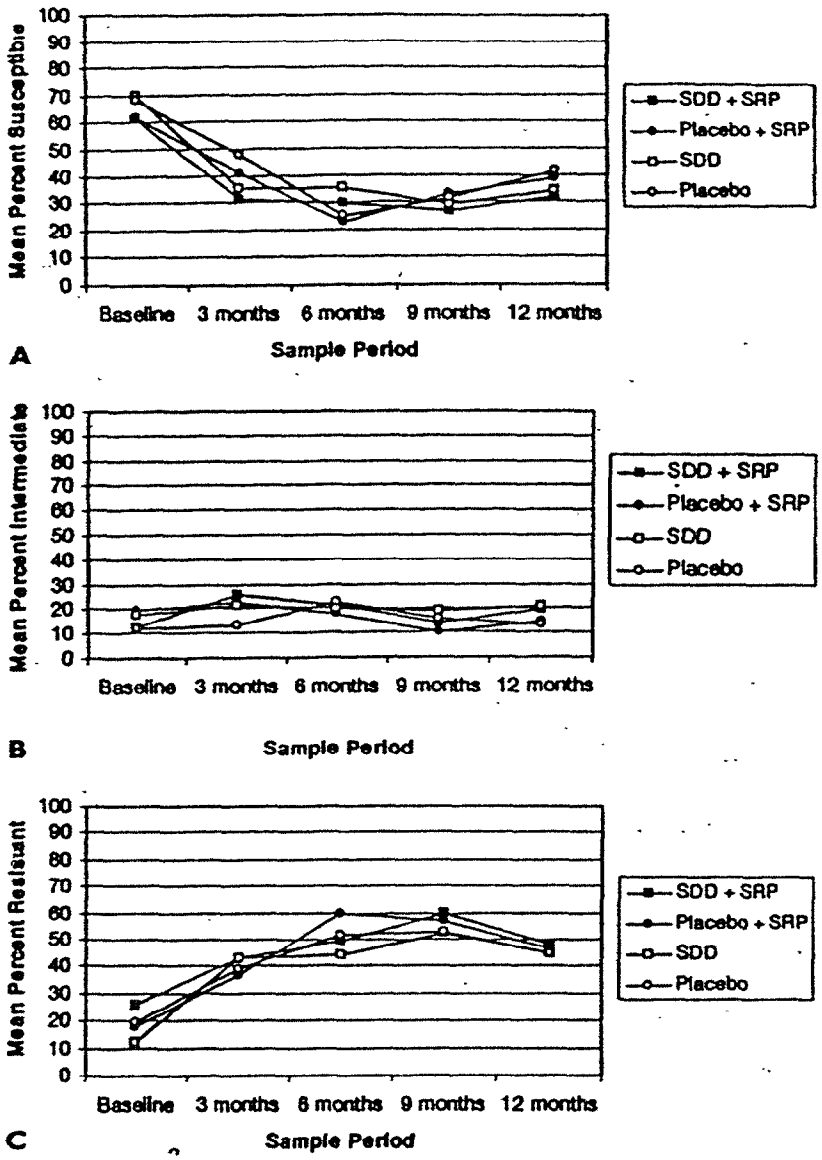

Figure 3.
A. Distribution of doxycycline-resistant isolates (shown as mean percent isolated) in the Susceptible category (≤ 4 μg/ml) by treatment group at each sample period.
B. Distribution of doxycycline-resistant isolates (shown as mean percent isolated) in the Intermediate category (5 to 15 μg/ml) by treatment group at each sample period.
C. Distribution of doxycycline-resistant isolates (shown as mean percent isolated) in the Resistant category (≥ 16 μg/ml) by treatment group at each sample period.

patients who received the placebo. Changes in MIC values in the Susceptible category seemed random. MIC values for *Fusobacterium* spp isolates remained low, thus precluding quantitative and statistical analysis.

Study 2 also demonstrated the lack of a positive correlation of cross-resistance with other antibiotics. Antibiotic profiles remained stable, as did cumulative MIC_{50} values independent of doxycycline dosage. There was no compelling evidence that MIC values were influenced by SDD administration, and MIC_{50} values did not suggest that resistance to SDD was developing over time. Changes in MIC values by sample period were most likely random. No evidence was found to suggest that the model for selective resistance following use of therapeutic antibiotics was in practice.

These studies emphasized the MIC_{50} values in conjunction with the MIC_{90} values for comparison because the latter are significantly influenced by outliers when small numbers of patients are enrolled in a study. Furthermore, with the small incremental changes measurable with the antibiotic-impregnated strips, subtle changes were magnified that would not have been recognized using the standard 2-fold dilution assay. The antibiotic-impregnated strips measure a 10,000-fold concentration, and establish a continuous gradient enabling qualitative accuracy, precision, and reproducibility reported within 0.5 dilution steps. In fact, an encouraging result was the stability of the antibiotic patterns and the reproducibility of the MIC values. Traditionally, emerging antimicrobial resistance is based on trend analysis, focusing on semi-quantitative susceptibility categories (Susceptible, Intermediate, or Resistant) or MIC_{50} values over time. The studies reported here used different methods (agar dilution and the antibiotic-impregnated strips) in different patient populations and analyzed by different laboratories, yet they reached the same conclusions. No evidence was found supporting developing antimicrobial resistance over time, and the changes in MIC values by sample period were most likely random.

Following the initial experience of studies 1 and 2, studies 3 and 4 were conducted with adequate power to assess whether significant differences in susceptibility could be attributed to SDD therapy compared with placebo. Furthermore, these studies sought to examine the influence of the drug on all the constituents of the subgingival microflora, rather than selected genera. These more rigorous studies showed no statistically or microbiologically significant differences between treatment groups in terms of the distribution of doxycycline-resistant strains, the predominant doxycycline-resistant taxa recovered, or evidence of multi-antibiotic resistance (defined by resistance to two or more unrelated antibiotics).

The correlations that were found between doxycycline resistance and resistance to minocycline and tetracycline were expected, as were the correlations between resistance to erythromycin and clindamycin. One mechanism of resistance to doxycycline or minocycline is due to ribosomal protection and is

encoded by a *tet* gene, which conveys resistance to all tetracycline derivatives (e.g., tetracycline, minocycline, and doxycycline). Similarly, resistance to clindamycin is encoded by an *erm* gene that also conveys resistance to erythromycin. Additionally, no cross-sectional or longitudinal differences were detected in the normal flora, levels of periodontal or opportunistic pathogens, and composition of doxycycline-resistant bacteria present.

SDD in combination with mechanical procedures has been shown to be effective in improving probing depths and attachment levels above improvements seen with mechanical procedures alone. Studies demonstrated that adult periodontitis patients who received SDD as adjunctive therapy to mechanical procedures showed significant improvements in probing depths and attachment levels over those who did not receive SDD as adjunctive therapy.[6,7,24,25]

In conclusion, the results of these four studies indicate that long-term SDD does not alter or contribute to alterations in the antibiotic susceptibility of the subgingival microflora compared with a placebo.

ACKNOWLEDGMENTS

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EXHIBIT G

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R#

Effect of Sub-antimicrobial Dose Doxycycline (SDD) on Intestinal and Vaginal Flora.

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Previous studies have demonstrated that a 9-month regimen of SDD (20-mg doxycycline, b.i.d.) did not exert a discernible effect on the composition of the subgingival flora or result in an increase in antibiotic resistance. The purpose of this study was to determine if SDD (20-mg, b.i.d.) had an effect on either the intestinal or the vaginal microflora. Seventy periodontally diseased subjects were entered at each of two centers (35/center), Ohio State University (OSU) and the University of Pittsburgh (Pitt), and randomized to receive SDD or placebo over a 9-month period. Stool specimens and vaginal swabs were collected at baseline, 3, and 9-month evaluations and shipped by overnight courier to the microbiology labs at UF. Each sample was examined for total anaerobic counts, opportunistic pathogens, and doxycycline-resistance (≥4 µg/ml). Doxycycline-resistant (DR) colonies were enumerated and a representative of each predominant colony type, up to 3, was subcultured, identified, and the susceptibilities determined to 6 antibiotics (doxycycline, minocycline, tetracycline, amoxicillin, clindamycin, and erythromycin). The microbial data from the two centers were combined for statistical analyses. Although the number of vaginal samples received was limited, no treatment differences were detected ($p>0.15$). No statistically significant differences were detected for the fecal samples. However, the total DR counts recovered at 3 months tended to be higher in the SDD treatment samples from Pitt ($p=0.08$) but not from OSU ($p=0.37$). This difference may have been due to the low number of specimens received from the placebo group at Pitt which unbalanced the sample. A similar trend was not apparent at 9 months (Pitt $p=0.37$; OSU $p=0.61$). *Bacteroides, Prevotella, Fusobacterium, Bifidobacterium, Clostridium,* and *Eubacterium* were the predominant DR genera recovered. There were no differences in the recovery of these genera either between or within treatments during the study. In conclusion, there was no evidence that SDD treatment exerted an effect on the composition or resistance level of either the fecal or the vaginal microflora.

Supported by CollaGenex Pharmaceuticals, Inc.

F#

MICROBIOLOGY REPORT

Protocol # DERM-301

Efficacy of Dermastat® (doxycycline hyclate) 20 mg tables administrated twice daily for the treatment of acne

Clay Walker, Ph.D.

Acne vulagaris is a self-limited skin disorder that is primarily associated with adolescents and young adults. The exact etiology is unclear but appears to be multi-factorial and involves the hypercornification of the pilosebaceous duct, an increase in sebum production, colonization with *Propionibacterium acne* and possibly other skin bacteria, followed by the subsequent production of inflammation. The resulting inflammation is a host immune response to the bacterial infection and leads to the production of both inflammatory and non-inflammatory lesions. Various antimicrobial agents have been used to lessen the effect of inflammatory acne. In this country, the tetracyclines are widely used. These antibiotics are generally given systemically at relatively low dosages for long periods of time. The effect of the tetracyclines appear to be exerted by interfering with the chemotaxis of PMNs and by scavenging oxygen radicals produced by the inflammatory cells rather than by an antimicrobial effect on the bacteria involved.

In the study described within, a sub-antimicrobial dosage of doxycycline (SDD) consisting of 20 mg doxycycline hyclate was given twice daily for a period of 6 months. The clinical objective of the study was to compare the effect of SDD relative to a placebo on the acne lesions. The microbial objectives were to determine (1) if SDD has any detectable antimicrobial effect on the normal skin flora, (2) leads to the overgrowth or colonization of the skin by opportunistic pathogens, and/or (3) results in an increase in antibiotic resistance by the predominant skin microflora.

Previous studies using SDD in the treatment of adult periodontitis, failed to detect any antimicrobial effect on the oral microflora or on the flora associated with the large intestines or the vagina. Since the etiology of acne is somewhat similar to adult periodontitis in that its involves an inflammatory response to the causative bacteria, SDD may provide a beneficial clinical response without exerting an antimicrobial effect.

METHODS

Subjects. A total of 50 subjects were entered into a double blind, placebo-controlled, 6-month trial to determine the effect of doxycycline, 20 mg bid, (SDD) on moderate facial acne.

Sample collection. Microbial samples were collected from the surface of the skin from a 2 cm^2 area in the center of the brow at baseline and after 6 months of treatment. The sample was collected by placing a 2 cm^2 template over the area to be sampled and then gently rubbing a sterile cotton swab over the area. The swab was placed in a tube containing 1.0 ml of pre-reduced, anaerobically sterilized (PRAS) Ringers solution. The tube was labeled with subject number and sample period and immediately transported to the microbiology labs for processing. Upon receipt in the labs, the sample tube was gently sonicated using a water-filled cup horn and a series of 10-fold dilutions was performed in PRAS Ringers fluid by use of a VPI Anaerobic Transfer unit to avoid introducing air into the sample. An 0.1 ml aliquot of each appropriate dilution was applied to the surface of agar media and spread with a sterile glass rod.

Sample processing. The sample was plated on non-selective media to determine the total number of anaerobic and facultative bacteria recovered. The sample was also be plated on the same non-selective medium containing 4 μg of doxycycline per ml for the isolation of doxycycline-resistant bacteria. Both the number of anaerobic bacteria and the number of facultative bacteria resistant to ≥4 μg of doxycycline was determined and expressed as a percentage of the total respective flora. The number of different doxycycline-resistant colonies were examined and a representative of the 3 (if present) most predominant doxycycline resistant colony types were subcultured from the anaerobic and the facultative plates. These isolates were identified to *genus* and *species*, where possible, and then tested for susceptibilities to 6 antibiotics (doxycycline, minocycline, tetracycline, erythromycin, clindamycin, and vancomycin). The first five of these antibiotics are frequently used in the treatment of acne;

vancomycin was tested to determine if either vancomycin-resistant streptococcus or staphylococcus might be present on the skin.

The microbial media used are given in Table 1 along with incubation conditions and confirmatory tests for the target microorganisms.

Table 1. Target microorganisms, media, incubation conditions, and confirmatory tests for the recovery and enumeration of microorganisms from the surface of the skin.

Target microorganisms	Medium	Incubation conditions	Confirmatory tests
Total anaerobic counts	Trypticase soy blood agar (TSBA)	Anaerobic, 37°C, 5-7 days	None
Total facultative counts	TSBA	10% CO_2, 37°C, 3-5 days	None
Total doxycycline resistant counts (anaerobically)	TSBA-doxycycline	Anaerobic, 37°C, 5-7 days	None
Total doxycycline resistant counts (facultative)	TSBA-doxycycline	10% CO_2, 37°C, 3-5 days	None
Propionibacterium acne	TSBA	Anaerobic, 37°C, 5-7 days	Colonial and cellular morphology
Enterics	McConkey's agar	Aerobic, 37°C, 2-3 days	Colonial & cellular morphologies
Staphylococcus species	Mannitol salt agar	Aerobic, 37°C, 2-3 days	Colonial & cellular morphology
Streptococcus species	Mitis-Salivarius agar	Aerobic, 37°C, 2-3 days	Colonial & cellular morphology, hemolysis
Gram positive rods	TSBA	Anaerobic, 37°C, 5-7 days & 10% CO_2, 37°C, 3-5 days	Colonial & cellular morphology, gram stain

Colony counts. Following the prescribed incubation period, the plates were examined for colony-forming units (CFUs). Total anaerobic counts and total facultative counts were determined from the plate dilution that gave rise to 30-300 CFUs. For all other media, colony counts were taken from plates with 30-300 CFUs if available. If less than 30 colonies were present on the most diluted plate, the actual colony number present was counted providing that more than a single colony was detected. A single colony on a plate was considered a "0" count.

Doxycycline-resistant colonies. The number of colonies resistant to 4 μg of doxycycline per ml was determined as described above. The proportions present were determined relative to both the total anaerobic counts and the total facultative counts. When available, a representative of each resistant colony type was subcultured, and identified to genus and species level by GLC analyses of cellular fatty acids. If present, up to 3 different colony types were subcultured. The proportion that each colony type contributed to the total doxycycline resistant flora recovered was calculated. Following the identification process, antibiotic susceptibilities were determined for each isolate, which survived the identification procedure, to 6 antibiotics by agar dilution methodology.

Statistical testing. Differences between the SDD treatment and placebo were sought using the unpaired t-test. If the data did not follow a normal distribution, the nonparametric Mann-Whitney test was used to avoid the bias of outliers. Differences within treatments were sought using a paired t-test or a rank sum test. A p value of ≤ 0.05 was considered as statistically significant.

RESULTS

A total of 50 subjects were entered at the University of Florida with 35 completing the study. Of the 35, 18 were in the SDD group and 17 were in the placebo group. The first group entered consisted of 30 subjects. Two additional groups of 10 each were entered later at the sponsor's request. The majority of the dropouts occurred in the latter two groups and particularly in the last group of 10 subjects. These drops were not product related but were primarily due to changes in the subjects' plans. Most were students at the University of Florida and elected not to attend the summer session. Several drops in the placebo group occurred due to the subject's perception that his or her acne was getting worse.

Microbial counts. . The means of the counts for each target group are given in Figure 1. No statistical significant differences ($p \leq 0.05$) were detected for any microbial group enumerated either between the SDD and placebo treatment groups or within either the SDD or placebo treatment group using the unpaired t-test for differences between groups and the paired t-test for differences within groups. The p-values are given in Table 2.

Table 2. P-values obtained from statistical testing using the t-test

Target group	BL SDD vs BL Placebo	6-mo SDD vs 6-mo Placebo	BL SDD vs 6-mo SDD	BL Placebo vs 6-mo Placebo
Total AnO2 Counts	0.4005	0.9032	0.3069	0.0902
Total CO2 Counts	0.8650	0.0896	0.2408	0.4192
AnO2 Doxy Res Counts	0.2792	0.4997	0.4908	0.6625
CO2 Doxy Res Counts	0.6864	0.7794	0.1991	0.1666
P. acne	0.4179	0.3276	0.3501	0.1755
Enterics	0.3320	0.2750	0.4027	0.2337
Gm + Rods	0.1691	0.2916	0.1413	0.7444
Streptococci	0.3324	none present*	0.4027	none present*
Staphylococci	0.0799	0.1640	0.2868	0.2686
% Doxy Res (AnO2)	0.7248	0.1967	0.3937	0.2178
% Doxy Res (CO2)	0.4872	0.0625	0.5525	0.1327

*Insufficient number of streptococci in 6-mo placebo group for statistical testing

Antibiotic susceptibilities. The major bacterial colonies that grew on medium containing 4 µg/ml of doxycycline were subcultured, identified, and subjected to antibiotic susceptibility testing to doxycycline and 5 other antibiotics. From some subjects, no colonies were detected that were resistant to doxycycline and in others only a single colony type was seen. Antibiotic susceptibilities were performed by agar dilution methodology over a concentration range of 0.25 to 32 µg/ml in two-fold dilutions to doxycycline, tetracycline, minocycline, erythromycin, clindamycin, and vancomycin. The results were reported as the minimal inhibitory concentration (MIC) required to inhibit visible growth on the agar medium. An MIC_{50} and an MIC_{90} were calculated for each bacterial taxa for each group at each sample period. Since no significant microbial differences were seen in the taxa recovered anaerobically compared to facultative, these data were combined. These values are given in Table 3 for doxycycline.

Table 3. MIC_{50} and MIC_{90} values for doxycycline for each treatment group

	SDD-baseline		Placebo-baseline		SDD-6 months		Placebo-6 months	
Taxa	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
P. acnes	32	>32	32	>32	16	>32	>32	>32
Staphylococci	>32	>32	32	>32	32	>32	16	>32
Diptheroids	8	16	4	16	16	32	16	16
Gram neg rods	32	>32	>32	>32	16	>32	>32	>32
Other	32	>32	32	>32	TFTC*	TFTC	TFTC	TFTC

*TFTC: Too few to calculate MIC_{50} and MIC_{90}

Microbially, there were no differences either between the treatments or within the treatments in the MICs obtained for doxycycline. A two-fold difference in an MIC value may yield statistical significance; but, by definition, a two-fold difference is considered within the error range when performing antibiotic testing.

Correlation testing was performed to determine if any strong positive correlations ($r \geq 0.70$) existed between resistance to doxycycline and any of the other five antibiotics tested. The correlation coefficients generated are given in Tables 4a-d.

Table 4a. Antibiotic correlations for SDD at baseline

	Doxy-cycline	Tetra-cycline	Mino-cycline	Erythro-mycin	Clinda-mycin	Vanco-mycin
Doxycycline	1					
Tetracycline	**0.5391**	1				
Minocycline	0.4606	0.2260	1			
Erythromycin	0.5255	0.2720	0.3292	1		
Clindamycin	0.3903	0.4956	0.2657	0.3319	1	
Vancomycin	-0.0009	0.0249	0.4993	0.1561	0.1458	1

Table 4b. Antibiotic correlations for SDD at 6 months

	Doxy-cycline	Tetra-cycline	Mino-cycline	Erythro-mycin	Clinda-mycin	Vanco-mycin
Doxycycline	1					
Tetracycline	0.1355	1				
Minocycline	0.4065	0.1712	1			
Erythromycin	0.2778	-0.1146	0.1972	1		
Clindamycin	0.2632	0.2822	0.2840	**0.6777**	1	
Vancomycin	0.0812	-0.0480	0.2788	0.3411	0.4066	1

Table 4c. Antibiotic correlations for Placebo at baseline

	Doxy-cycline	Tetra-cycline	Mino-cycline	Erythro-mycin	Clinda-mycin	Vanco-mycin
Doxycycline	1					
Tetracycline	**0.5208**	1				
Minocycline	0.2584	0.2759	1			
Erythromycin	0.2345	0.1483	0.2500	1		
Clindamycin	-0.0728	-0.0642	0.3209	**0.5156**	1	
Vancomycin	-0.5298	-0.4598	0.1325	-0.2978	0.1018	1

Table 4d. Antibiotic correlations for Placebo at 6 months

	Doxy-cycline	Tetra-cycline	Mino-cycline	Erythro-mycin	Clinda-mycin	Vanco-mycin
Doxycycline	1					
Tetracycline	0.1258	1				
Minocycline	**0.5325**	0.2147	1			
Erythromycin	-0.2692	-0.1513	0.1302	1		
Clindamycin	0.2344	-0.1581	0.4073	**0.6977**	1	
Vancomycin	0.1872	-0.2268	0.1202	-0.4120	-0.0276	1

Examination of the correlation values obtained indicate that there were no strong correlations ($r \geq 0.70$) present. The strongest correlation was between erythromycin and clindamycin ($r = 0.5$ to <0.70), which was expected since most bacteria that are resistant to clindamycin are also resistant to erythromycin. Moderate, but not strong, correlations ($r=0.5$) were detected between doxycycline and tetracycline and minocycline in some instances. Again, this was expected since many bacteria with resistance to one tetracycline are frequently resistant to the other tetracyclines as well due to the carriage of tetracycline resistant genes coding for ribosomal protection.

There were no apparent differences in the correlation coefficients obtained for the SDD at 6-months relative to either the placebo 6-month samples or to the SDD-baseline samples.

Bacterial taxa with resistance to doxycycline. The subcultures described above were identified to genus and species where possible. The bacteria recovered with doxycycline resistance belonged primarily to the staphylococci, diptheroids (a loosely defined group of gram positive bacteria from the skin), and assorted gram-negative facultative rods. A few *P. acne* were recovered with resistance to doxycycline. However, none of the streptococci or enterics from the skin demonstrated doxycycline resistance. The major groups recovered were expressed as a percentage of the total doxycycline isolates recovered and were then compared between and within treatments to determine if a significant microbial shift had occurred. These data are expressed graphically in Figure 2.

CONCLUSIONS

Treatment of subjects with moderate acne with a 6 month regimen of SDD exerted no detectable effect, either statistically or microbially, on the microbial skin flora relative to either baseline values or to 6-month placebo values.

These data may be summarized as follows:

1. There was no change in the composition of the normal skin flora
2. There was no increase in the proportion of the cultivable flora resistant to doxycycline
3. There was no increase in MIC values obtained for the bacteria resistant to 4 µg of doxycycline per ml.
4. There was no microbiologically or statistically significant change in the composition of the cultivable flora with resistance to 4 µg of doxycycline per ml.
5. There was no evidence of the development of cross-resistance between doxycycline and related or unrelated antibiotics.

Based on these data, the treatment of moderate acne with a 6-month regimen of SDD exerts no detectable antimicrobial effect on the cultivable skin flora.

Fig 1. Means of the target organisms enumerated

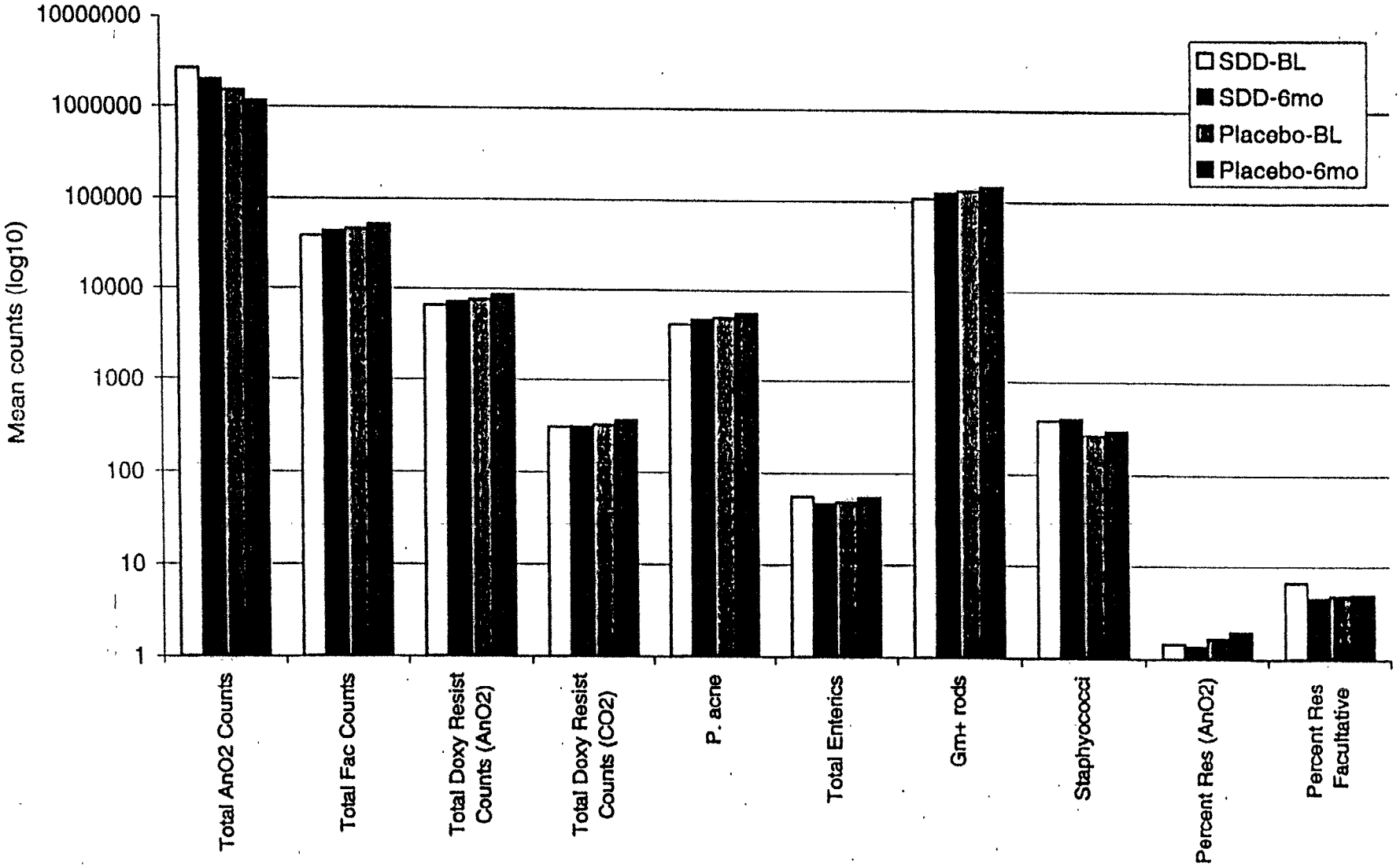

Fig 2. Doxycycline-resistant taxa recovered and respective pr